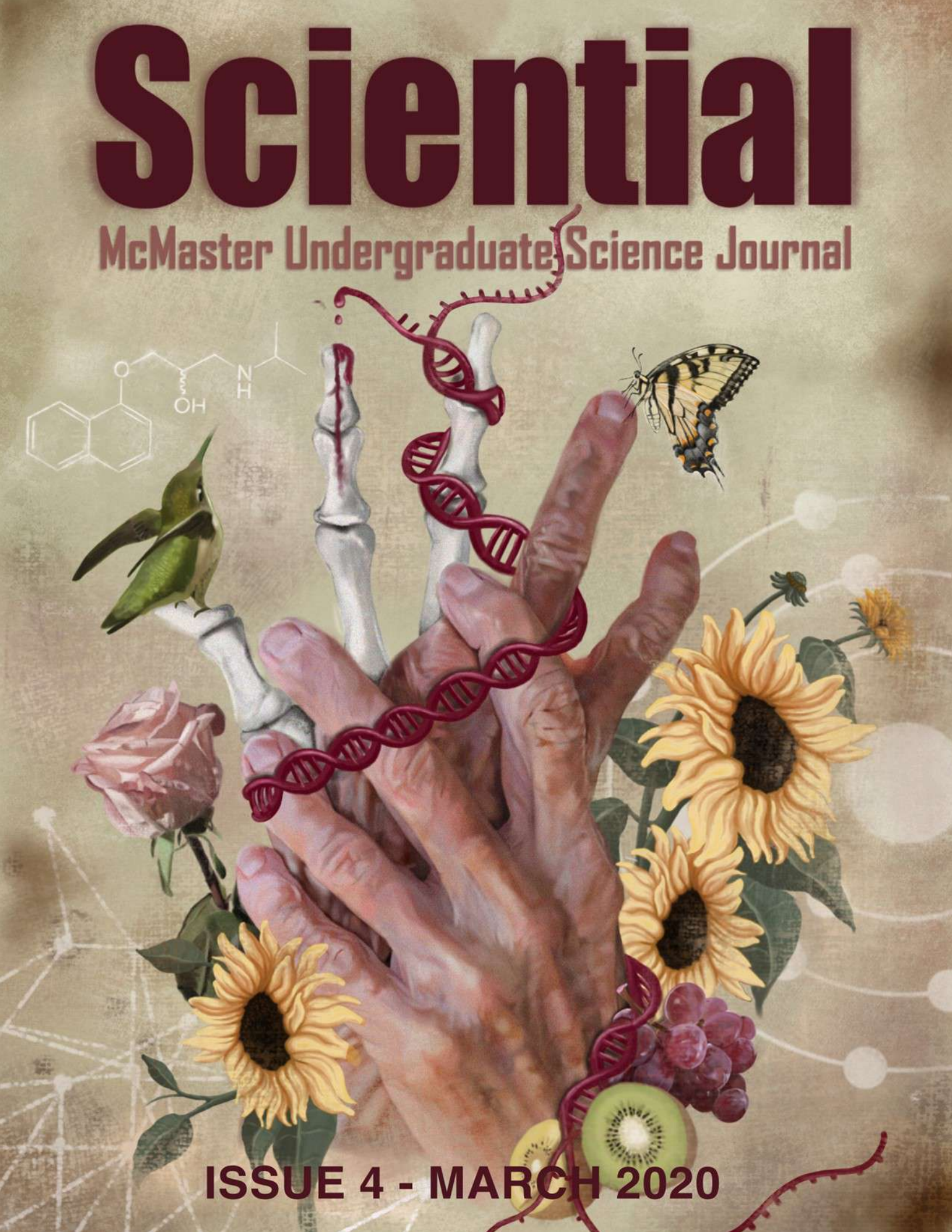
# Sciential

## McMaster Undergraduate Science Journal



ISSUE 4 - MARCH 2020

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# DEAR READER,

Welcome to Issue 4 of *Sciential*! The global pandemic has been an unnerving, mournful, and a hindering, yet educational experience for the world. We take unsurmountable inspiration from the hard work of our healthcare workers and the solidarity communities are exercising to ensure our wellbeing. Meanwhile, the *Sciential* team has been deliberate in presenting you with our most exquisite of submissions. Here, we explore a versatile range of scientific disciplines, from virology, to mental health, the origins of life, touching upon acute and chronic immune disorders, and exploring health and climate change in the context of dietary choices.

Issue 4 commences with a literature review by Hannah Mahoney addressing the central theories of the origins of life and highlighting the significance of this field in the contemporary world and scientific research. Following, a review manuscript by Pouriya Sadeghighazichaki, Tara Sabzvari, and Ava Oliaei discusses the implications of meat-based diets on health and climate change and pitches the necessity for a transition to plant-based alternatives. Succeeding, Milena Hurtarte and Kasia Tywonek introduce a novel immune-based therapy for Acute Lymphoblastic Leukemia while discerning its mechanism of action, ongoing clinical trials, and therapeutic potential in the near future. Following, we are presenting the COVID-19 infographic authored by Youssef El-Sayes displaying the current knowledge base on the virus, including its origins, testing options, and available antiviral treatments. Succeeding, Reza Khorvash and Ram Upadhyaya conducted an interview with Dr. Tobias Berg shedding light on his work in developing therapeutics for Acute Myeloid Leukemia as well as his career as a clinician and a scientist. The News and Views section opens with a piece authored by Alisa Nykolayeva and Aiman Shahid reporting on the novel biomarker of Major Depressive Disorder and suggests it as a diagnostic and perhaps a therapeutic tool for depression. Succeeding, with infinite pride we are thrilled to feature a News and Views piece by Stefano A. Biasi et al., who deliver a discussion of a treatment regiment as an early intervention measure in Rheumatoid Arthritis. Finally, the issue closes with an Opinion Piece authored by Isabel Dewey and Caitlin Reintjes on the bioethical considerations of gene editing while discussing the scientific and philanthropic limitations of implementing gene editing into contemporary health care practice.

We are optimistic that you will enjoy the range and the relevancy of the submissions in this issue. This release is a fruit of deliberation and expertise of our authors and our extraordinary Editorial and Creative boards who facilitated a seamless publication process and assisted the authors with suggestive review style and graphical appeal. We are especially grateful for the marvelous work ethic and talent of our Senior Editors, Amama Khairzad and Ishita Paliwal, and Creative Director, Youssef El-Sayes for excellent directing and guiding the publishing process. It is also our surmount pleasure to acknowledge our Senior Advisor Team, Dr. Kimberley Dej, Dr. Veronica Rodriguez Moncalvo, Dr. Katie Moisse, and the exceptional Science Librarian, Abeer Siddiqui for their remarkable instruction and support in delivery of every issue of *Sciential*. Finally, we are grateful to our generous sponsors, Science Initiative Fund (SIF) from McMaster Science Society, who bolster our efforts in delivery of our publications to you, our reader. In veneration of the *Sciential* team, we sincerely hope that you will enjoy Issue 4 and that you stay safe and well in these unprecedented times.

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ACADEMIC REVIEW OPEN ACCESS

# The Origins of Life: The Metabolism First, Replication First, and Compartmentalization First Theories


## ARTICLE INFORMATION

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## ABSTRACT

When, where, and how did life on Earth originate? The origin of life problem involves multiple scientific disciplines and research that dates back several decades to the early 1900s. The origins of life can be summarized into three hypothetical stages: (1) the origin of biological monomers, (2) the origin of biological polymers, and (3) the emergence and evolution of cells. While highly speculative, the connections between these stages are theorized by attempting to determine the geochemical conditions which could have facilitated the emergence of specific chemical functions of biological systems. This literature review summarizes some reported findings that are relevant to the early Earth environment and the main theories in regard to the origin of life. Specific focus is placed on the Metabolism First, Replication First and Compartmentalization First theories. These theories are relevant to the origin of life paradox, which concerns whether metabolism or RNA was the first aspect of life to form. Understanding the processes that encouraged the emergence of life can lead to advancements in drug discovery and allow for a deeper understanding of ecological processes. Overall, the aim of this literature review is to discuss the origin of life theories and highlight the importance of future research in this field.


**Keywords:** Origin of life, early earth, interdisciplinary science

## INTRODUCTION

The origin of life (OOL) is a scientific problem that spans multiple disciplines, such as chemistry, biology, and thermodynamics, and includes many theories that will be discussed in depth. It investigates the source of life on Earth, specifically the natural processes that allowed organic life to arise from non-living matter. While it is generally agreed that life arose from a single primitive life form, there is little evidence that demonstrates how this occurred.[1,2] A highly speculative field of study, the OOL has been debated since the early 19th century.

Life's emergence from non-living matter is poorly understood. There is a consensus among the scientific community that nature evolved from non-living matter through a step-by-step process.[3] The OOL scientific community is focused on determining these steps. To do this, researchers examine the chemical and environmental conditions necessary for the emergence of life.

The conditions in the early Earth environment could have allowed for the occurrence of prebiotic chemistry and the emergence of biologically relevant molecules. Although there are current theories of how life arose, they consist of some paradoxes that need to be discussed. Overall, the OOL theory is vital for understanding life today.

## EARLY EARTH ENVIRONMENT

The early Earth environment plays an important role within the OOL theory. One of the first people who theorized the OOL was Harold Urey, who proposed

that life arose from countless natural experiments of increasing complexity that involved many different combinations of molecules, light, and energy.[1,2,3] Despite many attempts to understand these processes, there is still much speculation. One part of the problem is that these processes would have occurred on a young and primitive Earth, whose characteristics were vastly different from those of the present.[2,3] Understanding the environment where such processes would have occurred is essential to answering questions about how they occurred. However, early Earth coincides with the end of the Hadean era and the beginning of the Archean era (Figure 1), an eon that has little evidence to indicate what the environment was like.[3,4] Current theories aim to describe the conditions found on early Earth and the resulting prebiotic chemistry.

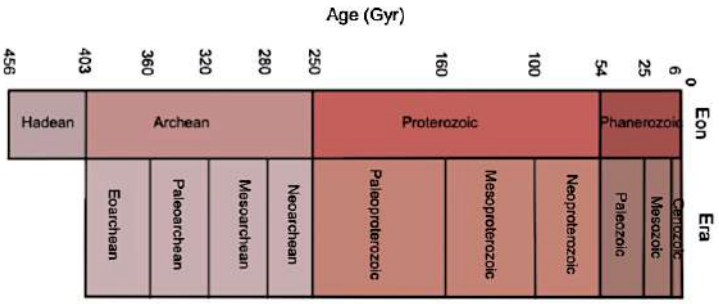


**Figure 1.** A geologic time scale representing the time from the formation of the Earth until the present. It is believed that life arose during the end of the Hadean era and the beginning of the Archean era.[4]

In 2002, the earliest evidence of life was discovered in the form of fossilized stromatolites that date back to 3.45 billion years ago (Gya).[5] Stromatolites are rock-like structures formed by cyanobacteria. The age and simplicity of the fossilized organisms suggest that the origin of life occurred at the beginning of the Archean Era, between 4 and 2.5 Gya.[5] Older stromatolite fossils were discovered in 2017. These fossils were tubular microorganisms found in iron and silica-rich rocks dating back to 4.28 Gya.[6,7] This discovery suggests that the origin of life could have occurred at the end of the Hadean Era, from 4.54 to 4 Gya. According to the geologic time scale (GTS), the Hadean Era covers the formation of Earth, the moon, and the primitive oceans and landmasses.[8] Overall, these fossilized remains support the general assumption that life began between the end of the Hadean and the beginning of the Archean Eras.

## *Earth's Early Atmosphere*

Some of the strongest evidence for the characteristics of early Earth have been identified through the use of detritus zircons.[9,10,11] Zircon ($ZrSiO_4$) is a mineral that is common in the crust of the Earth and can occur in all three rock types: sedimentary, igneous, and metamorphic.[12] Detrital zircon refers to sedimentary rocks which have been exposed by erosion.[10] Detrital zircons are highly durable, heat resistant, and provide information about the chemical characteristics of the environment from which they formed, as they have an extremely stable bonding structure.[10,11] This makes them useful in determining the characteristics of Earth during the Hadean Era. The discovery of detrital zircons in mid-western Australia has led to evidence suggesting that the first oceans and continental crust formed on Earth as early as 4.4 Gya, which falls into the Hadean Era.[12]

Experiments conducted in 1952 and 1953 by Stanley Miller and Harold Urey aimed to model the disequilibrium chemistry that would have resulted from electrical discharges, or ultraviolet radiation, being absorbed in highly reduced atmospheres consisting of methane, ammonia, and water.[1,2,13] The experiments were based on the conclusion that early Earth likely had a reducing atmosphere because of its high temperature and concentration of hydrogen. This would have reduced the likelihood of highly reactive free oxygen particles.[14,15] It was unlikely that the atmosphere contained more than trace amounts of carbon, nitrogen, oxygen, and hydrogen, other than as $CH_4$, $H_2O$, $NH_3$ (or $N_2$) and $H_2$.[2] This idea was supported by the Miller-Urey experiment in 1953, which tested the chemical origin of life under conditions stimulated to mimic those believed to be present on early Earth.[2] One-step reactions of the mixture components ($CH_4$, $H_2O$, $NH_3$, $H_2$) produced compounds such as HCN and $CH_2O$, which were then able to form biomolecules such as amino acids and glycine, as shown in Equations (1) and (2), respectively.[1,2] The experiment was able to produce 11 out of the 20 known amino acids and was part of the first chemical evidence of abiogenesis, or life emerging from inorganic substrates.[1,2] In 2007, scientists re-examined sealed vials from the original experiments and identified more amino acids than previously reported, classifying more than 20.[16] The discovery of more amino acids is linked to increased efficiency in detection equipment and procedural techniques.

$$\begin{aligned} CO_2 &\rightarrow CO + O^* \\ CH_4 + 2[O] &\rightarrow CH_2O + H_2O \\ CO + NH_3 &\rightarrow HCN + H_2O \\ CH_4 + NH_3 &\rightarrow HCN + 3H_2 \end{aligned} \quad (1)$$

$$CH_2O + HCN + NH_3 \rightarrow NH_2\text{-}CH_2\text{-}CN + H_2O \quad (2)$$
$$NH_2\text{-}CH_2\text{-}CN + 2H_2O \rightarrow NH_3 + NH_2\text{-}CH_2\text{-}COOH$$
(glycine)

More recent evidence published in 2011 by Trail et al., has suggested that early Earth's atmosphere may not have been as reducing as originally thought at the time of the Miller-Urey experiment.[17] Major volcanic eruptions during early Earth would have released nitrogen

($N_2$), carbon dioxide ($CO_2$), hydrogen sulphide ($H_2S$) and sulphur dioxide ($SO_2$) into the atmosphere, and likely played a critical role in determining its composition.[17,18] Identities of molecular species in magmatic outgassing, the release of gas from volcanoes, depend on the partial pressure of oxygen. The partial pressure of oxygen depends on the magmatic characteristics of the volcanoes.[17] Volcanic melts that have oxygen partial pressures close to that defined by the iron–wüstite buffer, a signature in the rock that indicates it has little oxygen, would yield volatile species similar to that of the Miller-Urey atmosphere ($CH_4$, $H_2$, $H_2S$, $NH_3$ and CO).[17,18] Conversely, melts close to the fayalite–magnetite–quartz buffer signify that the rocks would have had a higher oxygen concentration and would have been similar to present-day conditions with $H_2O$, $CO_2$, $SO_2$, and $N_2$ gases.[17] The oxidation state of samples from the Hadean era magmatic melts are consistent with that of the fayalite–magnetite–quartz buffer, suggesting that the atmosphere was less reducing.[17]

### *Earth's Early Oceans*

The climate of the Earth's oceans is an important component in understanding the emergence of life. Liquid water likely originated on Earth when it cooled enough for water vapour in the atmosphere to condense.[19] Evidence for the characteristics of such oceans is typically found using isotope ratios. This is a method of comparing the relative abundance of isotopes in samples, such as rocks and water, to elucidate the characteristics of the samples such as age.[19,20] Using the ratio of $^{18}O$ to $^{16}O$ isotopes found in fossils or rocks, researchers are able to determine the temperature at the time the organism existed or the rock was formed.[19] Using Equation (3), as seen below, the $\delta\ ^{18}O$ value corresponds to the ratio of both oxygen isotopes in the sample divided by the ratio of a standard, which is anything that has a known isotopic ratio.[19,20] Obtaining temperature estimates from oxygen isotopes is based on equilibrium isotope fractionation, the partial separation of isotopes at chemical equilibrium.[20,21] The heavier isotope,$^{18}O$, condenses in the liquid phase of water, and the lighter isotope $^{16}O$, is found in the vaporous phase of water. A $\delta\ ^{18}O$ above one indicates a higher number of $^{18}O$ isotopes, and therefore a cooler climate, while a $\delta\ ^{18}O$ value below one indicates a higher number of $^{16}O$ isotopes, and therefore a warmer climate.[19,20,21] The $\delta\ ^{18}O$ values of Archean marine sediments are tens of times lower than values today, suggesting a warmer environment.[19] A hot early Earth is supported by other evidence, with oceanic temperatures calculated to be approximately 70˚C.[20,21] As well, this result is supported by silicon isotope ratios which have been interpreted as showing evidence of ocean temperatures ranging from 60 to 80˚C.[21] Converting $\delta\ ^{18}O$ to a temperature value in degrees Celsius (˚C) is done using Equation (4), where correlates to the temperature in ˚C and correlates to the $\delta\ ^{18}O$ value computed with Equation (3).[19,20,21]

$$\delta O^{18} = \left( \frac{\frac{o^{18}}{o^{16}sample}}{\frac{o^{18}}{o^{16}standard}} - 1 \right) * 1000\% \quad (3)$$

$$T = 16.5 - 4.3\delta + 0.14\delta^2 \quad (4)$$

The pH of the Earth's early ocean is highly debated due to opposing experimental evidence. In 2013, Blatter et al. interpreted Archean calcium ($Ca^{2+}$) isotopes to reflect high $Ca^{2+}$ alkalinity ratios, suggesting that an alkaline ocean with a high $pCO_2$ value was unlikely.[22] On the other hand, Friend et al. in 2008 argued the existence of an alkaline ocean due to the presence of alkaline earth elements (e.g. $Mg^{2+}$ and $Ca^{2+}$ isotopes) that corresponds with high pH environments.[23]

## THE ORIGIN OF LIFE

Life is generally characterized by three requirements: (1) the ability to reproduce, (2) the ability to evolve, and (3) the presence of metabolism.[24] In present living organisms, these functions are operated by biopolymers, such as DNA and RNA, proteins, and phospholipids in highly balanced, syndicate systems that have evolved over billions of years.[24,25]

The OOL theory proposes that life began in the form of simple elements.[1,2] Through chemical evolution, identifiable structures like amino acids, nucleotides, and fatty acids formed.[1,2,13] These early molecules developed the ability to perform actions that mimicked modern metabolism, replication, and evolution.[1,25,26] Eventually, they converged to form the last universal common ancestor (LUCA).[26] Overall, the origin of life can be summarized into three basic stages: (1) the origin of biological monomers, (2) the origin of biological polymers, and (3) the evolution from molecules to cells which are able to metabolize, reproduce, and evolve.[26] While this statement is a general outline for the OOL, there is little scientific evidence which connects these steps.[1,2,26] For this reason, many theories have arisen to suggest which aspect of life could have emerged first.[1,26]

A popular theory is the 'prebiotic soup theory', proposed in 1929 by Alexander Oparin.[1,2,27] This theory suggests that life arose in the 'primordial soup' of the early Earth environment and that organic molecules in the soup reacted in an increasingly complex fashion.[27] While recent research has changed what was hypothesized about the early Earth environment since the theory's proposal, the soup theory remains central to the scientific community's understanding of the OOL.[27] Three of the most current popular theories are: (1) the Metabolism First Theory, (2) the Replication First

Theory, and (3) the Compartmentalization First Theory. The following sections will consider the evidence for each theory.

## METABOLISM, RNA, OR COMPARTMENTALIZATION FIRST?

Metabolism is a chemical process that occurs within a living organism in order to maintain life.[25,28] In present-day organisms, metabolic systems employ enzymes, receptors, biomolecules, and cofactors that react in various ways to create energy to fuel life processes.[28] Life today also has genetic material, RNA and DNA, which are blueprints for producing proteins and enzymes.[28] Finally, life can also be characterized by compartmentalization and the ability of eukaryotic cells to regulate energy conversion and ion concentrations with the help of lipid membranes.[29] These three aspects are essential for life to occur and would have been required for the LUCA to arise, but the order in which these aspects developed is highly debated.

The debate in which aspect of life arose first is commonly referred to as the 'origin of life paradox'.[30] For life to have arisen, there must have been a genetic molecule, RNA or DNA, capable of transferring blueprints for making effective proteins and enabling the ability to pass on changes to the next generation.[31] Nevertheless, RNA and DNA cannot function without the help of proteins to replicate, transcribe, and translate.[31] As well, these reactions require some form of a compartment to separate them from other molecules that could affect the process. However, membranes that act as compartments in cells today are made of lipids that require proteins to be synthesized.[31] Therefore, while the following sections outline some of the evidence supporting each theory, the question of which aspect of life came first cannot be answered definitively. The emergence of these aspects could have happened in conjunction with each other, or in a different way that has not yet been theorized.

### *Metabolism First Theory*

The Metabolism First Theory suggests that self-sustaining networks of metabolic reactions may have been the first forms of simple life.[26] This idea of self-sustaining networks was first introduced by Dr. Stuart Kaufmann, who proposed the idea of autocatalytic sets as a method for the emergence of life. Autocatalysis is the phenomenon where a group of chemicals react and create products that catalyze their own formation, thus creating a self-sustaining set (Figure 2).[26]

Initial chemical reaction pathways could have produced more complex chemicals that were then able to catalyze their own formation.[26] Eventually, these react

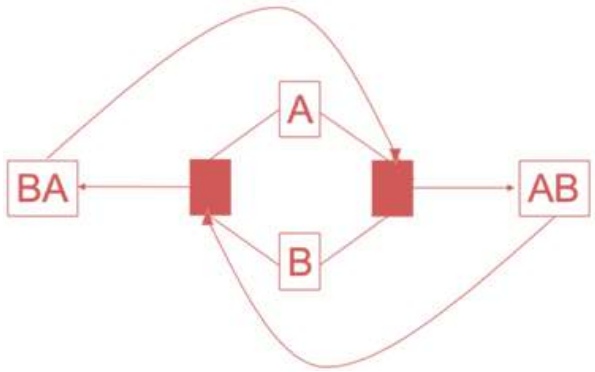


**Figure 2.** A simple autocatalytic set in which monomers A and B combine to form polymers AB and BA, which are then able to act as enzymes to catalyze their own formation (represented by the filled boxes).[32]

-tion networks could output recognizable products, such as amino acids. Once these sets were formed, molecules that could have acted as some form of genetic material would have been formed. The formation of membranes to enclose and concentrate these sets would have been produced subsequently. Kaufmann also proposed ways in which these sets could reproduce and evolve. Theoretically, if the constituents of each set were to double, two identical sets could form, offering a rudimentary version of reproduction.[26] Overall, the Metabolism First Theory employs theoretical biology, computational systems, and mathematical concepts to theorize a way in which life could have emerged.

### *Replication First Theory*

The Replication First Theory suggests that life first arose as self-replicating nucleic acids, such as RNA and DNA.[33] It is commonly accepted that RNA was more likely the first genetic material for several reasons.[34,35,36] Since RNA contains ribozymes, used to catalyze chemical reactions, it could potentially catalyze a reaction and copy itself.[37,38] The discovery that RNA could act as both a carrier of genetic information and a catalyst provides a solution to the protein/genetic material paradox that was previously mentioned.[30,31] The enzyme would be able to produce additional copies of itself, which would continue to multiply until the supply of constituents that would be used to create nitrogenous bases and other building blocks were exhausted. Mutations could have arisen, some of which could have been beneficial with respect to the replicase function. Therefore, the evolving population of RNA enzymes could have developed the ability to replicate certain substrates with increasing efficiency.[39] After the creation of RNA, other elements such as metabolic networks or compartments could have formed.

One problem with the Replication First Theory is a lack of evidence to substantiate it. Laboratory evidence for the spontaneous assembly of oligonucleotides ex-

ists, but it is limited in the scope of temperature and reliability.[40] This is due to the tendency of proteins to denature at high temperatures (~41˚C).[41] In experiments using the water-soluble functional group carbodiimide as the condensing agent, short oligonucleotides were formed but the reactions were slow and inefficient.[39,40] Another problem arises from the relative complexity of ribozymes, which cannot self-assemble without the presence of a polymer backbone.[41]

### *Compartmentalization First Theory*

The Compartmentalization First Theory refers to the introduction of a simple cell membrane or other methods of compartmentalization occurring as the first step towards the OOL.[42] The theory questions how any chemical reaction, whether forming molecules or reaction networks, could have arisen if there was no way to concentrate constituents.

The belief that there needed to be a concentrating mechanism for life to arise cannot be singularly attributed to the Compartmentalization First Theory. It has been suggested that there had to be some mechanism of concentrating necessary elements and molecules for prebiotic chemistry to occur and for life to emerge.[27, 43, 44] Alexander Oparin suggested that liquid droplets, formed at the bottom of Earth's early ocean due to differences in water density, could have contained a high enough concentration of constituents to eventually turn into living cells.[45] Other theories have suggested that perhaps iron-sulphide bubbles, which were compartments that formed on the ocean floor near hydrothermal vents, could have acted as primitive membranes at life's origin.[45] They could have grown by inflation due to the hydrostatic pressure of the fluid from the external environment, as well as by osmosis catalyzed by organic and inorganic molecules trapped within the bubbles.[45, 46] The idea that life may have started in membrane-less micro-droplets, like iron-sulphide bubbles, has been highly researched and is generally an accepted theory.[29] While life could have started in membrane-less micro-droplets, eventually, a simple organic membrane would have needed to develop, as modern-day cells are characterized by a bilipid membrane.[29, 47, 48] Modern-day cells are made of amphiphilic lipid molecules that self-assemble into a membrane when exposed to a polar environment.[47, 48]

Self-assembly in mixtures of a single species has been heavily investigated in the last two decades, as it offers a simple explanation for the spontaneous emergence of lipid membranes for the OOL.[29] Groups of similar fatty acids lead to a more efficient self-assembly process under a broad range of conditions. However, some problems arise with more complex mixtures of amphiphilic material, such as fatty acids of different lengths and constitutions. While the mixtures will still self-assemble into aggregate structures, their half-life is four to nine times shorter than that of vesicles formed from single type mixtures.[29] Additionally, they have a smaller aqueous volume enclosed in the vesicle, which would affect how well these membranes concentrate inorganic and organic substituents.[47] On the other hand, complex mixtures of fatty acids and their derivatives are considered more likely to have constituted the source pool for compartmentalization processes at the OOL.[29] This is because it would be very unlikely for only one type of fatty acid to be outputted initially, and for the reaction to be stable enough to output a large enough number of these fatty acids to form a membrane.[29, 47] The consequences of this, along with the lack of evidence confirming the emergence of a supportive framework, have led to uncertainty about the Compartmentalization First Theory. As mentioned previously, the lifespan of vesicles formed from complex mixtures is shorter than that of single type mixtures.[29] In general, longer lifespans of molecular products and structures increases the probability that they will react with other molecules and form the next step in the OOL.[29] This duality suggests that more evidence is required to elucidate some of this theory's inherent complications.

## CONCLUSION

Will it ever be possible to determine the exact moment when life arose? Most likely not. The answer to the question of when life arose is limited to fossilized evidence of microscopic organisms; this enables the window of time for when life could have arisen to be narrowed, but it has yet to offer any definitive evidence of the LUCA.

Although it will likely never be known *when* life on Earth first arose, it is still important to research *how* life on Earth arose. Life is built on several molecular components and understanding how these components have formed helps to answer some of the questions around the nature of these building blocks. Therefore, a deeper understanding of the OOL has applications to genetic therapy, drug development, and cures for diseases that require an understanding of biological systems.[49, 50, 51]

This literature review discussed the early Earth environment and some of the main theories underlying the OOL theory, specifically those pertaining to what aspect of life occurred first: metabolism, compartment, or genetic material. Due to the speculative nature of the OOL theory, the belief of how life emerged will continue to change with the discovery of new chemical mechanisms and evidence. Future research will hopefully be able to further the understanding of the geochemical inventory of Hadean Earth and how it would be able to fuel the processes and stages involved in chemical evolution. Therefore, the OOL is an extremely complex puzzle in the scientific community. Future

research and discoveries could also significantly change or shatter some of the deepest held beliefs on how life originated. Nevertheless, with a complex problem comes a never-ending list of discoveries.

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ACADEMIC REVIEW OPEN ACCESS

# Assessing the Efficacy of Plant-Based Diets on Human Health and in Mitigating Climate Change

## ARTICLE INFORMATION

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## ABSTRACT

Meat consumption and current livestock farming practices have a multitude of detrimental impacts on climate change and human health. Today, livestock farming is one of the largest contributors to greenhouse gas emissions (GHGs). The manure and chemicals used in livestock farms also seep into the water supplies and degrade the quality of water. Furthermore, livestock require a vast expanse of land for grazing and feeding, which leads to deforestation and habitat fragmentation. High meat consumption and its associated effects have also been implicated in causing various health complications in humans such as a higher prevalence of cardiovascular diseases, antimicrobial resistance (AMR), and an overall increase in mortality. Transitioning towards plant-based diets could not only mitigate the impacts of climate change, but it could also improve human health. This paper assesses the efficacy of transitioning towards plant-based diets and the overall benefits and challenges of this transition. This literature review is crucial as it compiles recent data about climate change and various studies about plant-based dietary transitions, as well as their impacts on the environment, human health, and climate change mitigation efforts.

**Keywords**: Plant-based diet(s), climate change, human health, consumption

## INTRODUCTION

Livestock production is one of the largest contributors to anthropogenic emissions and accounts for 60% of non-$CO_2$ and 16% of all $CO_2$ emissions worldwide.[1] Given the rising population, which is projected to reach 9.8 billion in three decades, and the subsequent economic growth, global calorie consumption is expected to double by 2050.[1] This is concerning as the current unsustainable and meat-derived food production and consumption model fosters the current climate catastrophe. Over 300 tons of meat are consumed each year worldwide, and this is contributing to an upward trend that is predicted to worsen over time.[2] It is important to note that meat production consumes more natural resources and energy when compared to plant-based foods.[2] Livestock production for food purposes is also a source of immense methane and nitrous oxide emissions, which account for 80% of all agricultural greenhouse gas (GHG) emissions.[3] This, in turn, contributes largely to climate change.[3] Currently, society is in dire need of an immediate change, which can be achieved by a wide-scale transition towards plant-based diets by consumers. Raising awareness about many different factors can be used to promote this transition and encourage individuals to incorporate this change into their diet. These factors include human health effects, animal welfare, and the deterioration of the environment through land degradation, air pollution, and water quality deterioration.[2] The Lancet Commission on Planetary Health stated that a transition towards a plant-based diet will allow for a significant reduction of these adverse environmental impacts.[4] This transition has already been shown to be attainable and effective in high-income countries. A study of this transition in 150 high-income countries found that a transition towards plant-based diets could lead to an 84% reduction in GHG emissions.[5] It is acknowledged that the complete elimination of meat-derived foods is not attainable due to various factors, such as cultural constraints. However,

progressively shifting towards these plant-based diets can allow for a significant reduction in GHG emissions and increase the sustainability of land and water use. This, in turn, can have both direct and indirect effects on human health.[6]

The potential of this dietary transition in mitigating the effects of climate change will be further explored in this paper. Many papers have studied the effect of a reduction in GHG emissions as a result of transitioning away from meat production. However, these studies have yet to assess the efficacy of this decrease in GHG emissions on diminishing climate change. Furthermore, with the introduction of dietary fads, such as the keto diet that endorse increased reliance on meat and animal products, this literature review aims to highlight the various negative impacts of consumption trends on depleting the environment and impacting climate change. This literature review analyzes research conducted on the feasibility and efficacy of a transition away from meat-based diets and towards those that are plant-based, as a potential for climate change mitigation. This paper will shed light on recent research available in the scientific community and delve further into how dietary changes on an individual level can have wide-scale effects on the environment and the current climate change problem. By looking to history and reverting to plant-dominated ancestral diets and consumption patterns, we may be able to reach a feasible solution to navigate the future of human civilization.

## EFFECTS OF LIVESTOCK FARMING AND FOOD CONSUMPTION TRENDS

### *Land Degradation*

One of the inevitable primary challenges associated with livestock production is land degradation through deforestation. A major contributing factor to this issue is the vast area of land that is required for maintaining livestock and farming practices. As the demand for meat-derived foods increases, the amount of land that is required will also understandably increase. This leads to deforestation, which is the leading cause of land degradation. This mass decrease in the number of trees evidently limits the amount of oxygen that is produced, while leading to an increase in carbon dioxide. According to a study done by Hansen et al. (2013), a 12 -year period from 2000 to 2012 found that 2.3 million square kilometres of forest were lost, mostly due to wildfires and livestock production.[7] The same study mentioned that approximately 30% of the earth's surface area is covered with forests; however, this area is drastically decreasing.[7] The process of deforestation for the purpose of livestock production typically involves the burning of trees, contributing to the increase in GHG emissions.[8] According to McMichael et al. (2007), the GHG emissions from agriculture and related land changes account for more emissions than the transportation sector and the generation of power.[9] The same study found that most of these emissions were caused by methane and nitrous oxide. These are gases that are also released due to the use of fertilizers and the manure of livestock.

Deforestation leads to a decrease in land area that could be used for the production of plant-derived foods.[10] Stehfest et al. (2009) compared cropland and grassland use in various reduced-meat consumption scenarios.[11] In reduced-meat scenarios for 2050 projections, a drop in ruminant meat consumption showed a decrease in grassland use per million hectares (ha).[11] Another study by Rosi et al. (2017) found that both vegan diets and ovo-lacto-vegetarian diets cause a significant decrease in global land usage per day, compared to that of omnivorous diets.[12] Keeping in mind the effects of livestock production on land degradation, it becomes evident that transitioning to plant-based diets can help to maintain land productivity, as well as reduce deforestation for grazing, and emissions from clearing.

### *Water Degradation*

In an agricultural setting, the main source of water quality degradation is through the leaching of chemicals and nutrients found in manure and fertilizers, into the water source. To explain, livestock manure is less dense in nutrients compared to commercial fertilizers. Primarily, manure contains a slightly different nitrogen to phosphorus ratio, where manure contains more phosphorus than nitrogen.[13] The nutrients in fertilizer are recognized as N-P-K, which are nitrogen, phosphorus and potassium, respectively. Each of these three nutrients contribute 16% to fertilizers with a ratio of 1:1:1, whereas manure has a ratio of 4:5:1 for N-P-K.[14] Since plants and agricultural productivity depend on nitrogen for crop yields, in addition to fertilizer, farmers may use excessive amounts of manure on their crops to supplement the lack of nitrogen in manure. This, in turn, can result in an over supplementation of accompanying phosphorous. Subsequently, this has its own set of negative implications as it leads to nutrient enrichment in surface waters.[14] Rainfall causes runoff, which in combination with the slope of the ground, causes livestock manure to find its way into surface waters. This causes eutrophication and adversely impacts the aquatic environment.[14]

Eutrophication takes place when algae and aquatic plant species absorb these excess nutrients and thrive.[15] However, in this process, the overgrowth of algae and plants deplete the oxygen in the aquatic environment and block the sunlight from reaching other organisms. As a result, aquatic organisms lack the resources required for growth and eventually die off.

Consequently, the decomposition of the dead algae leads to further depletion of oxygen in the aquatic environment. It is evident that manure causes significant disruption of various natural processes. Therefore, nitrogen and water management programs must be put into place and followed stringently to prevent these adverse outcomes. One example of these water management programs is the Livestock Manure Pollution Prevention Project, that was initiated by Environment Canada's Water Quality Working Group in 2014. This program educates and provides resources to farmers and livestock producers for good manure management practices.[16] Such programs cause additional costs and economic burden to the government and third-party corporations.

It is also important to note that there is a lot of variety in manure quality and composition depending on the livestock's diet, living conditions, and administered medications. The toxic waste material, chemicals and nitrates in manure from consumption of antibiotics and medications could leach into groundwater and result in long-term contamination of water bodies. One particular study by Carpenter (2005) found that antibiotics that were administered to livestock were detected in groundwater 40 years after antibiotic use.[17] As a result, the long-term consumption of antibiotic-rid waters by both humans and livestock leads to antibiotic resistance. This topic will be further explored in the health impacts section of this paper.

In summary, the aquatic environment and the quality of surface water and groundwater are all affected by agricultural activities and farming practices. This imposes both social and economic burdens on the government and society as a whole. As well, the dead and endangered organisms due to eutrophication pose a threat to the sustainability of fisheries, which many workers depend on for a living. The costs of management programs and personnel responsible for the enforcement of regulations is a big hurdle for the government.[18] Finally, high levels of nitrates that seep into the groundwater through soil can be toxic to both livestock and humans. This can in turn place pressures on our healthcare system and reduce the quality of life. Therefore, meat consumption can deteriorate the quality of water through livestock and agricultural chemicals, such as antibiotics and toxins found in manure.

## *Air Pollution*

Livestock farming has a big impact on air pollution, mainly through GHG emissions. It is a large contributor to the agricultural sector, which accounts for approximately a fifth of the total GHG emissions in the world.[6] Producing and maintaining livestock demands the burning of fossil fuels for energy, as well as deforestation for freeing up land for the animals. Both of these processes result in an increase in GHG emissions, which further promotes climate change.[5] Therefore, consuming more meat products will promote the burning of more fossil fuels and deforestation processes, which ultimately contribute to the worsening of the current climate catastrophe. McMichael et al. (2007) found that the agricultural sector was responsible for approximately 22% of the global GHG emissions, which is a greater contributor than the transport sector.[9] Transitioning towards a diet that abides by the World Health Organization's (WHO) recommendations consists of greater consumption of fruits and vegetables and reduced consumption of meat products. This will allow for an estimated 17% reduction in GHG emissions.[19] Moreover, Rosi et al. (2017) found that both vegan and ovo-lacto-vegetarian diets contributed similarly to decreases in carbon footprints, while omnivorous diets led to increases in carbon emissions.[12]

Lastly, Westhoek et al. (2014), found that decreasing livestock production by halving the consumption of meat products and livestock derived foods, will cause an approximate 25-40% reduction in GHG emissions.[20] It is evident through these studies that livestock production is a large contributor to air pollution, and thus, climate change. A transition towards plant-based diets is essential in order to help the environment from further deterioration.

## *Health Impacts*

Due to intensive livestock farming and the infectious disease burden in developing countries, there is a massive use of veterinary antibiotics.[21] In developing countries, such as India and China, livestock farmers often misuse and abuse antibiotics to prevent the onset of illness for their livestock. Therefore, antibiotics are the most extensively used drugs in these countries, outweighing human antibiotic use. This may be due to the fact that there is typically a lack of education, standards, and enforced regulations regarding antibiotic use in these third-world countries. [22] These contributing factors cause an exacerbation of the antibiotic issue in these regions, which leads to the rise in antibiotic resistance, or antimicrobial resistance (AMR). [23]

This antibiotic-containing manure, as mentioned before, will find its way into aquatic and terrestrial ecosystems and disrupt the natural flora and fauna, in addition to affecting human health.[24] Thus, easily treatable infectious diseases may cause human mortality and morbidity due to the ineffectiveness of antibiotics. This places an increased economic burden on the healthcare system and the quality of treatments delivered since more treatments are required to counteract the effect of AMR and treat an illness.[25] A lack of access to treatment would subsequently reduce the quality of life of individuals and cause a financial burden on those affected. Additionally, this could reduce the

productivity of the livestock sector as animals will not respond as well to the antibiotics and may spread the infectious disease to other animals. [25]

Infectious diseases are disorders that are contagious and can be passed from one person to another. An example of a transmittable infectious disease is methicillin-resistant Staphylococcus aureus (MRSA). In a study by Graveland et al. (2011), the researchers found that MRSA was able to transmit between humans and animals due to its ability to adapt to new hosts. [26] They found that the main contributing cause of this was poor hygiene and excessive antimicrobial use, which initiated MRSA in farm animals.[26] This was then transmitted to the farmers, through direct contact with the animals. The virus was also transmitted to other humans through the consumption of infected meat, which then developed into infections, which are treated with effective antibiotics.[26] Evidently, antibiotics are used to treat, not prevent, infectious diseases. Excessive use of antibiotics reduces the efficacy of these medications, which leaves humans and animals alike, defenseless against transmittable infectious diseases.[26]

To overcome the AMR issue, which can ultimately be transmitted to humans who consume meat products and subsequently cause illnesses, the Government of Canada has implemented a new regulation.[27] As of December 1st, 2018, all Medically Important Antimicrobials (MIAs) used for veterinary purposes require prescriptions. [27] This is a significant development in mitigating the antibiotic resistance issue in North America. These initiatives are also necessary for developing countries who are leading the AMR crisis. This initiative indirectly supports a transition to plant-based and other meat alternative food products, which would not contribute to the issue of antibiotic resistance.

Another health impact of meat consumption is the increased risk for cardiovascular disorders. A study conducted by the European Union (EU) in 2018, found that halving the meat and dairy production would lead to a decrease in livestock farming. They also noted that these dietary changes would result in a decrease in saturated fat consumption, which would then lead to a decline in cardiovascular disease-related deaths.[27] Red meat contains cardiovascular risk factors, including blood lipids and lipoproteins. Substituting red meat with high-quality plant protein sources, but not with fish or low-quality carbohydrates, leads to more favourable changes in blood lipids and lipoproteins.[28] Cardiovascular disorders are not the only chronic non-communicable disease that meat consumption may instigate. A study published in Nature in 2014, found that type II diabetes incidences are greatly increased due to our current high meat consumption diet and may lead to a reduced life expectancy.[29] Another study conducted in the United Kingdom (UK) by Milner et al. (2015), found that if the average person's diet in the UK was adapted to conform to the WHO's recommendations, it would increase the average life expectancy by eight months.[29] As demonstrated in Figure 1, various diets are linked with different non-communicable chronic illnesses. Specifically, a vegetarian diet results in the biggest reduction in the relative risk of type II diabetes.

Another chronic non-communicable disease is cancer, which has been linked with excessive meat consumption.[30] Increases in protein intake are known to increase the amount of nitrogenous residues entering the colon. This can then result in N-nitrosation by the bacteria in the colon.[30] A study by Hughes et al. (2001) discussed how this process may have the potential to contribute to cancer formation, specifically colorectal cancer.[30] In summary, meat is a staple ingredient in the global diet, however, it has a multitude of adverse health outcomes that can be remedied by transitioning away from meat-centric diets. [30]

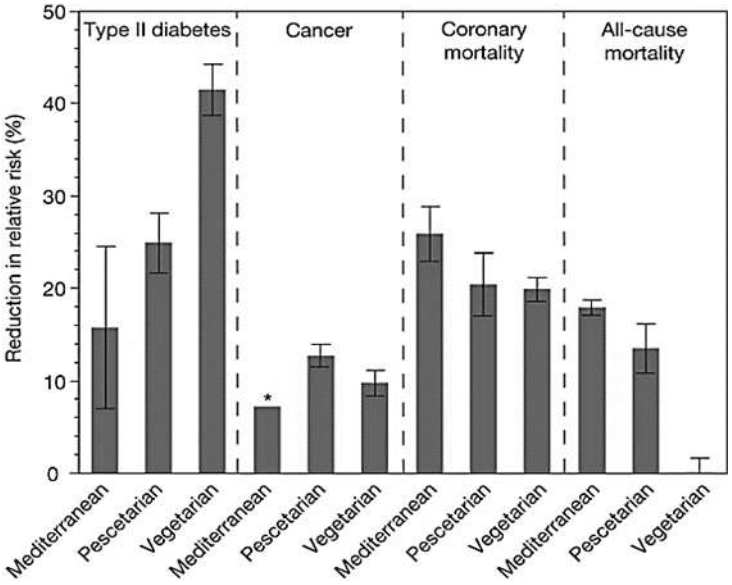


**Figure 1. Percent relative risk reduction associated with each disease mortality for each diet type.** Reprinted from "Global diets link environmental sustainability and human health", by Tilman & Clark (2014), *Nature*. In this figure, the x-axis represents the three types of diets evaluated in this study: Mediterranean, pescatarian and vegetarian. The y-axis represents the percent reduction in the relative risk of cardiovascular disease. This figure demonstrates a percent reduction in relative risk of cardiovascular disease in individuals with type II diabetes, cancer, coronary mortality and all-cause mortality for each of the three diets. The error bars represent the variability in data throughout the sample population. As portrayed in the figure, the greatest percentage drop of relative risk for cardiovascular disease was seen in the vegetarian diet for type II diabetic individuals.

## PLANT-BASED ALTERNATIVES

On August 8, 2019, a special report from the Intergov-

ernmental Panel on Climate Change (IPCC) highlighted plant-based diets as a major opportunity for mitigating and adapting to the impacts of climate change if the world's population adopted a variety of diets.[31] The efficacy of this solution is mainly associated with the reductions in GHGs, with plant-based diets and even diets with moderate meat consumption demonstrating significant emission reduction potential.[32] The complete elimination of animal-sourced foods was projected to decrease GHG emissions by almost eight gigatons of $CO_2$ annually.[32] This highlights the fact that plant-based diets that eliminate animal-sourced foods have vast mitigation potentials when it comes to climate change. What is worth noting is that other, more moderate changes, which may be easier to implement, can also benefit the environment and mitigate climate change significantly. For instance, diets with meat or seafood once a month and limited meat and dairy consumption were projected to decrease GHG emissions by six and five gigatons respectively.[32] There was also a substantial climate change mitigation potential from diets moderate in meat consumption that primarily consist of vegetables, leading to an annual reduction of three gigatons of $CO_2$.[32] Raising livestock for consumption is intrinsically an inefficient process due to the fact that they are at a higher trophic level than plants, and as a result there is a loss of energy. Therefore, sustaining livestock requires a vast amount of resources with fractionally less food output.[3] Livestock farming utilizes natural resources such as plants and grains that could otherwise be used as a food source for humans.[3] Comparatively, Westhoek et al. (2014) in the EU found that cutting meat, dairy products, and eggs by half can decrease GHG emissions by 25-40% and decrease per capita land use by 23%.[20] Animal feedlots are the largest contributor of nitrogen to the environment, and transitioning away from meat also demonstrated significant improvements to water quality in the EU.[20]

Research conducted by McMichael et al. (2007) proposed that reduced meat-consumption is better than the complete elimination of meat from human diets.[9] They state that it may not be possible to eradicate meat altogether; nevertheless, cutting back on meat production and consumption will improve both human and environmental health. They specifically suggested an international contraction and convergence strategy. Since global food consumption patterns are highly varied, with some developing countries undernourished and some first-world countries overnourished, it is important to evenly assign quotas for meat consumption. The study found that currently, on average, the global consumption of meat is 100 grams per person per day, not considering the variations between high-income and low-income countries. Contrarily, if this amount was changed to 90 grams per person per day, composed of 50 grams of red meat specifically, reaching global targets for climate change mitigation is likely. These include emission reduction targets as well as the enforcement of policies and changes that promote climate change mitigation (i.e. Emission taxation, and Cap and trade). McMichael et al. (2007) also sheds light on the fact that climate change is not only affecting land, air, food and water directly. It is also affecting biodiversity and elemental cycles, such as nitrogen fixation and carbon storage. To ensure a significant reduction in global emissions of $Co_2$ per year in gigatons by 2050, multiple strategies must be implemented. However, it is evident that among the different strategies, the flexitarian diet results in the least amount of emissions produced.[33]

Although there are various environmental benefits of plant-based diets, there are a lot of anticipated challenges when making this transition. Meat has been viewed as the highest quality of protein source, which in addition to its taste, makes this product very appealing to consumers.[34] There have been a lot of advancements in the production of plant-based meat alternatives; with products entering restaurants, fast-food chains, and supermarkets. Soybean proteins, texturized vegetables, and countless other plant proteins have been successfully utilized to create meat analogues.[34] From a consumer perspective, these analogues are also desirable due to the health image they convey as they are cholesterol-free, of lower cost, and provide a similar taste and protein makeup as animal-based meats.

The significance of this dietary transition resides in its mitigative potential for climate change, as well as other associated risk factors such as various health impacts, diminished land, air, and water quality. Research conducted by Springmann et al. (2018) used an integrated environmental and health modelling framework to assess consumption trends in more than 150 countries.[5] They found substantial reductions in GHG emissions of up to 84% when transitioning to plant-based diets.[5] Two of the substantial models they used included a 25-100% reduction in animal-sourced foods to plant-based foods and a flexitarian diet based on public health objectives to include balanced food consumption.[5] Figure 2 demonstrates the global mitigative potential of a complete elimination of all animal-sourced foods. This dietary change was projected to reduce total emissions from the studied countries by approximately 80%. There were other associated benefits including reductions in premature mortality, cropland use, nitrogen and phosphorus application.[5] It is important to note that the income of a country played a role in the reduction potential in these various areas. Higher-income countries, due to their increased resiliency and access to resources, can adapt much more quickly than lower-income ones, allowing more efficient transitions that minimize resource use. Low-income countries have a reduced ability to adapt to change due to lower access to resources and technology, failing to maximize benefits due to inefficiencies along the way.[5] Despite the socioeconomic varia-

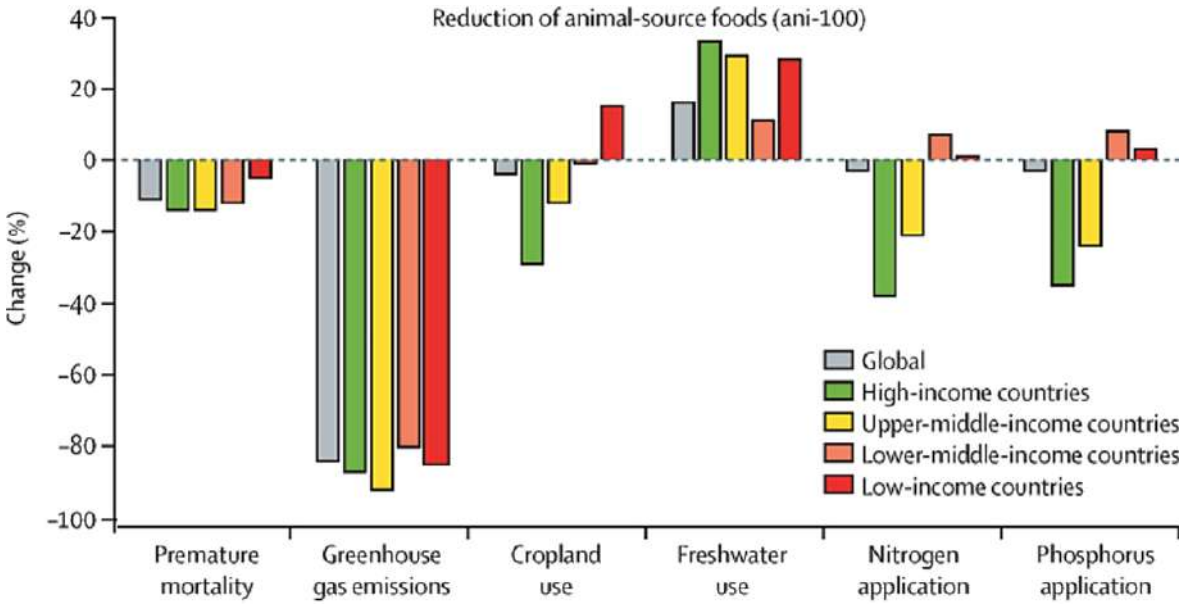


**Figure 2. Mitigation potential of the complete elimination of animal-based foods.** Adapted from "Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail", by Sprimgmann et al. (2018), *The Lancet Planetary Health*. In this figure, the x-axis represents the various factors evaluated in this study that impact animal-sourced foods. The y-axis represents the percent change in different criteria in response to the complete elimination of animal-sourced foods (ani-100); negative values indicate a reduction. As portrayed by the figure, GHG emissions represent the greatest percent reduction in animal source foods across varying socioeconomic statuses (SES) unlike other categories that demonstrated both increase and decrease depending on the SES. Although on average there is a decrease in premature mortality, cropland use, nitrogen and phosphorus application, freshwater use seems to increase in this scenario.

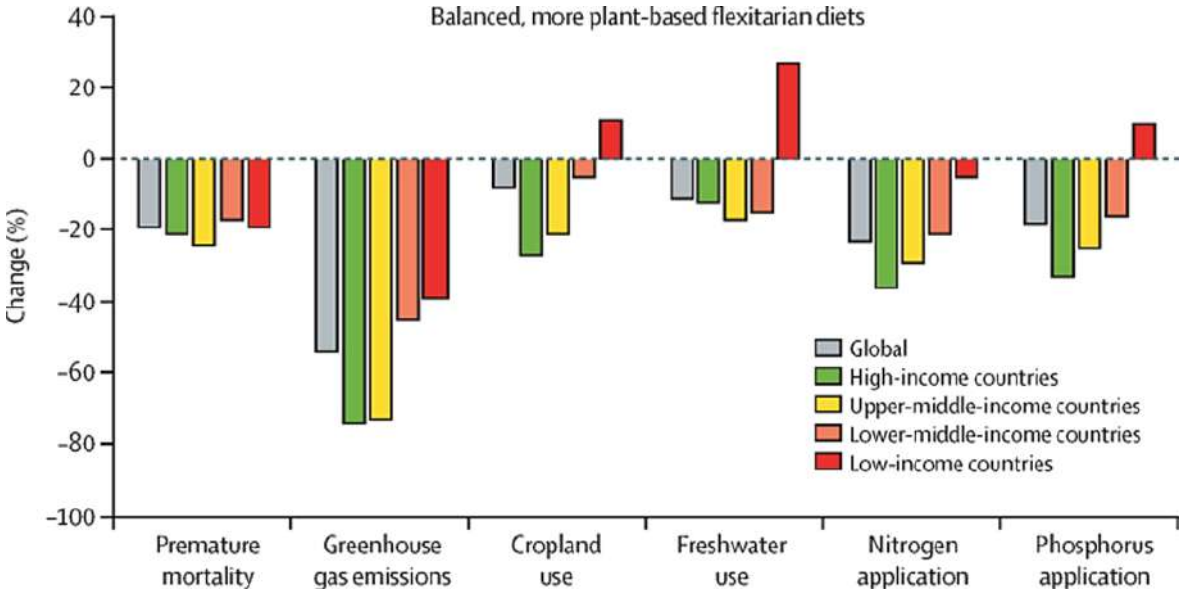


**Figure 3. Mitigation potential of balanced diets including animal-based foods.** Adapted from "Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail", by Sprimgmann et al. (2018), *The Lancet Planetary Health*. In this figure, the x-axis represents the various factors evaluated in this study that impact animal-sourced foods. The y-axis represents the percent change in different criteria in response to balanced flexitarian diets that rely more on plants; negative values indicate a reduction. As portrayed by the figure, GHG emissions represent the greatest percent reduction in animal source foods across varying socioeconomic statuses (SES). On average there is a decrease in premature mortality, cropland use, nitrogen and phosphorus application, and freshwater use. Low-income countries demonstrate an increase in cropland use, freshwater use, and phosphorus application.

tions of high and low-income countries, the GHG emission reduction was approximately the same across all income levels. This demonstrates the crucial role that unsustainable consumption plays in inducing climate change, as well as the feasibility of implementing this solution on a global scale.[5] Figure 3 demonstrates reductions in the negative impacts of global diets, similar to Figure 2. What distinguishes Figure 3 is that this assessment followed public health objectives and flexitarian diets that consist of a balanced consumption of animal-sourced foods (i.e. different meats and animal products), with plants comprising the majority of consumption. Figure 3 highlights global reductions in premature mortality, cropland use, freshwater use, and nitrogen and phosphorus application. In fact, flexitarian diets demonstrated a greater reduction in premature mortality at 20% in comparison to the elimination of animal-sourced food scenario.[5] Moreover, flexitarian diets were also projected to improve freshwater use, which is something that was not observed in the case of complete elimination of animal products.[5] This is believed to be in part due to the higher volume of plants required to sustain caloric intakes globally.[5] Therefore, in some respects, balanced diets have a more pronounced benefit for the environment. Nevertheless, in regard to climate change, Figure 3 highlights the immense mitigative potential of balanced diets with a 58% reduction in GHG emissions globally, for the 150 countries studied.[5]

Overall, Springmann et al. (2018) demonstrated that high emission reductions, and therefore climate change mitigation, was possible across all income levels in the various countries that they analyzed.[5] Additionally, they were able to determine other potential benefits on human health and the environment with both the reduction of animal-sourced foods, as well as a transition to balanced diets that relied more on plants. This study not only helped support the environmental benefits of eliminating meat consumption but also established the mitigative properties associated with balanced diets. This is important as the complete elimination of meat from diets is more difficult to implement than balanced diets.

## LIMITATIONS

One limitation in promoting wide-scale transition to plant-based diets is more intrinsic. A study by Eker et al. (2019) contributes a different point of view in human consumption patterns.[35] The authors of this study acknowledge that meat reduction from the global diet is the solution to diminishing the effects of climate change. However, the researchers also considered the behavioural mechanisms and the motivators behind decision-making for this dietary shift, as well as its implications for the food system. They mention the 'social norm effect', which is the human desire for obeying the socially accepted behaviours established by society. This comes into play when discussing new and innovative diets. The authors state that the extent to which the population adopts a certain diet influences the rate at which it becomes a norm of society.[35] The study stresses that in order to see real change and adoption of climate mitigating diet changes, two things must happen: there needs to be an intrinsic drive to implement the diets and a group dynamic that will motivate this decision.[35] These personal identifying factors may trump decisions to improve human health and the climate crisis.[35]

In an attempt to mitigate the climate crisis, many studies are proposing solutions without considering the feasibility of the idea in terms of human motivation. A clear trend is evident in research suggesting that one method of reducing the effects of climate change is to change the human diet, by completely or partially eliminating meat from our foods. In reality, this method may not be implemented by society. A study by De Boer et al. (2013) assessed the psychological reasoning behind our decisions, particularly when it comes to diets.[36] The authors of this article found that in a Dutch sample population, individuals who cared more about nature were willing to adopt a meat-free meal plan in an attempt to help climate change mitigation. However, for those who did not even believe in this crisis, the idea was received negatively, and they did not value this diet.[36] In today's society, the population seems to be divided in their opinions on climate change. As a result, plans to overcome the climate crisis should consider external factors and the parties involved. As outlined, since motivation and beliefs are a big factor in determining the success of meat-free diets, climate efforts should consider this when devising their mitigation plans. The majority of studies conducted mainly focus on the quantitative aspect of this environmental catastrophe by accounting for costs, temperature, and land area. However, this anthropogenic issue requires human motivation in order to be put into place, which cannot be executed if humans are divided on the existence of the issue itself. Further, mitigation strategies that suggest the elimination of a food group that has been a staple in the human diet are not feasible if not viewed positively and with an intent to implement these changes.

Despite the strong support for the environmental benefits of dietary transitions to plant-based foods, further research is needed on the ingredients used in producing these meat analogues. Although they portray a healthy image, there needs to be more studies on the actual health benefits of plant-based meat alternatives by assessing the long-term impacts of consumption. There is also a need to improve the chemical makeup of these products, and their nutritional and functional properties. Additionally, there needs to be a greater effort to raise awareness and educate the public on the worsening climate crisis. As well, it is crucial to positively shape their perceptions on plant-based diets to intrinsically motivate society to implement change.

Based on the results of these studies, it is evident that food consumption patterns and livestock farming are crucial to climate change mitigation. Therefore, policies should be implemented to help enforce dietary changes through subsidizing plant-based food alternatives that often cost much more than animal-based foods for consumers. Additionally, more research is required to highlight both the environmental and health benefits of plant-based diets in order to help consumers make better-informed decisions. Climate change is one of the most important issues faced by humankind and it is important to investigate potential solutions like dietary changes, which can be implemented in a shorter time span and across the globe without reliance on future technological advancements as key methods of combating climate change.

## CONCLUSION

Current food consumption patterns are unsustainable due to the heavy reliance on animal-based foods and products. Furthermore, livestock farming is an intrinsically inefficient process that consumes high quantities of natural resources in comparison to its overall food output. Many sources have listed the global food industry as a significant contributing factor to climate change and have recommended changes to help combat the effects of food production on climate change.

As outlined in this paper, meat consumption has a wide array of negative effects on the environment. One major issue caused by meat production is the land degradation that livestock farming induces. Inefficient land use that is instigated by livestock grazing and vast areas that are deforested yearly to expand agricultural areas for feeding livestock are contributing to this problem. This further impacts the natural carbon capture of trees and exacerbates climate change. Furthermore, the manure and heavy use of pesticides, herbicides, and antibiotics, reduces the quality of nearby bodies of water. The processes involved also have a negative impact on air quality. As a significant contributor of anthropogenic emissions, livestock farming emits vast amounts of GHGs into the atmosphere. Lastly, research has demonstrated that livestock farming not only impacts the ecosystem but also has implications for human health as evidenced by the exacerbation of antibiotic resistance and increased prevalence of other diseases.

This review aimed to shed light on the potential of a transition to plant-based diets to mitigate the effects of climate change. Based on the countless environmental and health issues associated with livestock farming, plant-based alternatives may provide a solution for transitioning away from meat. Research has demonstrated the countless environmental benefits of plant-based diets and the mitigation potential for climate change through reduced emissions. Additionally, health benefits such as decreased mortality and cardiovascular issues, have also been linked to plant-based diets. There is also an increasing amount of meat analogues entering the market, which have the same taste profiles and protein makeup as meat. Given this information, a shift to plant-based alternatives is not only a solution that is beneficial for mitigating the impacts of climate change but is also feasible to implement on a smaller scale. However, this field requires further research to determine the overall feasibility of this transition on a larger scale, in order to have significant environmental benefits. Additionally, more policies and incentives need to be put into place by governments to improve the access and affordability of plant-based alternatives for mass consumption.

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Due to the nature of this study, there were no ethical considerations. This paper abided by the established guidelines of the Lancet journal for writing systematic reviews, to the best of its ability. The criteria for study inclusion consisted of studies conducted from 2001 to the present day when assessing the mitigation potential of plant-based alternatives and environmental degradation caused by meat consumption. This paper sought research with global projections and stimulations as well as small-scale estimates. We would like to also acknowledge Dr. Luc Bernier for all his efforts, guidance, and essential feedback. This research did not receive any funding. There were no conflicts of interest.

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ACADEMIC REVIEW OPEN ACCESS

# A Review on Anti-CD19 CAR T Therapy against B-Cell Malignancies and Future Implications


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## ABSTRACT

This review examines CAR T CD19 immunotherapy, a newly FDA approved targeted therapy for B-Cell Acute Lymphoblastic Leukemia treatment. This therapy utilizes modified T cells from the patient's immune system, engineered to possess an anti-CD19 receptor that can recognize the specific CD19 antigen expressed on the surface of malignant B-lymphocytes. Using this highly individualized treatment, cancer types with a high rate of metastasis or relapse can be treated by the targeted nature of this therapy. The review aims to summarize the process through which CAR T was developed, from its inception to FDA approval. The material examined is current until March 2019 and explores the mechanisms and management of CAR T cell toxicity experienced by patients undergoing treatment. Clinical trials from respective stages of development are also detailed and summarized. The viable treatment options for patients suffering from B-cell acute lymphoblastic leukemia (B-ALL) are outlined. Despite the promising remission rates of CAR T therapy, its accessibility is limited due to current cost of treatment. With advancements in technology and improved understanding of immune-based therapies, it is possible that this method can become a more viable and affordable treatment option for patients in the future.


**Keywords:** Immunotherapy, B-ALL, personalized medicine, CAR T therapy

## INTRODUCTION

### *What is B-Cell Acute Lymphoblastic Leukemia?*

Acute lymphoblastic leukemia (ALL) is a cancer which is caused by the transformation and proliferation of lymphoid type progenitor cells in bone marrow, blood and extramedullary sites.[1] B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive form of ALL characterized by the differentiation and proliferation of immature white blood cells, called B-cell lymphoblasts, in the bone marrow and in circulation due to chromosomal abnormalities and genetic mutations .[1] Statistical data from the American Cancer Society illustrates that ALL onset follows a bimodal distribution, affecting those under 18 and over 50 years of age.[2,3] There are many cytogenetic subtypes of ALL corresponding to a multitude of mutated or translocated genes which cause specific types of cancer.[4,5] The genetic variation decreases the effectiveness of general therapies when treating ALL. The specificity of each case of ALL is a foundational reason for the creation of immunotherapies such as CAR T that can be individualized to a patient's genetic history.[6]

### *Treatment Options for B-ALL*

As a result of various mutations expressing ALL and the large age range affected by the disease, additional research is necessary for the development of treatment options that considers the genotype, phenotype, and risks involved for individual patients.[1] Individualized treatment approaches vary, but consistently emphasize remission-induction therapy followed by intensification therapy and continuation treatment to maintain remission.[1,7,8]

Chemotherapy is generally the first course of action for

most treatment plans. It is referred to as the remission induction phase, in which 99% of the initial leukemia cells are eradicated without compromising the normal function of all systems.[8] The most commonly used chemotherapeutics are glucocorticoids, such as prednisone or dexamethasone which inhibit cell metabolism to slow tumour growth.[8] A second category of systemic therapeutic agents include tyrosine kinase inhibitors (TKI) which inhibit protein phosphorylation, therefore protein function, in order to slow tumour growth.[8] This treatment can be administered in conjunction with various chemotherapies as combination regimes.[9,10] This approach is a generalization because treatment plans vary, depending on the patient's condition and potential side effects of the therapy, despite the cancer's aggressive phenotype.[11,12] Older patients are not able to handle aggressive chemotherapy doses, therefore a regime is created using both chemotherapy and a TKI to reduce side effects while still attempting to reach the goal of remission induction safely.[8,9]

Once remission is achieved, the next stage is to ensure the cancer is controlled and no longer proliferating at a high level. This phase is referred to as the intensification phase. This phase includes high doses of methotrexate and asparaginase therapies.[9,13] These treatments tend to be around 20-30 weeks in length and consist of one or more anti-leukemic drugs at high doses to reinforce the current remission.[14] The intensification phase can also include allogeneic cell transplants which transfer stem cells from healthy individuals to cancer patients in order to replace the patient's immune system cells and more effectively fight the malignant cells.[5] In fact, 45-70% of long term survival rates of adult ALL can be attributed to transplantation, compared to 30-40% attributed to chemotherapy.[1,15,16]

The last phase of treatment is the continuation or preventative phase that requires prolonged treatment once the patient is in remission.[1] Patients can be treated with potentially two more years of chemotherapy as a long term option.[1] Another option is a daily combination regimen of methotrexate and mercaptopurine which interfere with cell growth, in order to ensure white blood cell counts are normal and the immune system is functioning normally.[1]

## PRINCIPLES OF CAR T CELL THERAPY

T cells are lymphocytic immune cells that protect the body from pathogens and malignant cells. The use of these engineered cells as a form of cancer therapy is a revolutionary scientific discovery, marking the beginning of a new era in medicine. This form of therapy utilizes programmed T cells expressing chimeric antigen receptors (CARs) on their surface to target tumour -associated antigens.[17] CARs are usually comprised of an extracellular domain consisting of an antigen binding moiety and a spacer, a transmembrane domain, and an intracellular domain that induces the signal transduction for T cell activation (Figure 1).[18] The extracellular antigen binding moiety is a single-chain fragment variable (scFV) derived from antibodies responsible for recognizing and binding to tumour-associated antigens (TAAs) expressed on the surface of malignant cells. The spacer component of the extracellular domain acts as the connection between the antigen binding moiety and the transmembrane domain. The transmembrane domain contributes to the stability of the receptor and anchors the CAR to the cell membrane.[18] The intracellular domain is mediated by co-stimulatory receptors that induce a signalling cascade to generate an apoptotic or necrotic immune response against malignant cells.[19] The components of the intracellular domain have evolved since the initial development of CARs in 1989 by Dr. Eshhar's group at the Weizmann Institute of Science, in Israel.[19] Currently, there are three generations of CARs comprised of variable co-stimulatory receptors within the intracellular domain.[20] The CAR T structure is dynamic, in that it has evolved since its discovery, and will continue to change with improved understanding through research on its use and future applications against solid tumours.

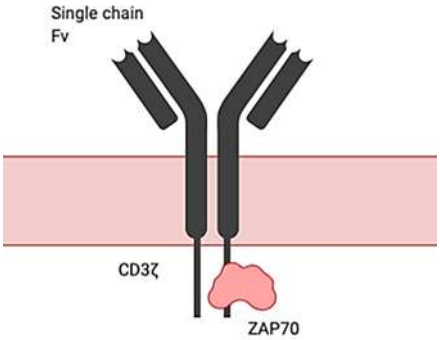


**Figure 1.** The model is a visual representation of the chimeric antigen receptor structure. The extracellular domain consists of the scFv from a monoclonal antibody that recognizes a TAA. The hinges and transmembrane domains are used to link the recognition domain and the intracellular signalling domain consisting of the CD3-ζ chain and co-stimulatory signalling molecules. The CAR structure is shown in complex with the signalling molecule Zap70. Image adapted on Biorender.[21]

### *Procedure*

The inoculation of CAR T- cells in patients is a complex, multi-step process shown in Figure 2. First, T cells are collected from the patient. Autologous T cells are collected by apheresis, a procedure that withdraws blood from the body. T cells are isolated and the remaining blood components are deposited back into the body.[22,23] Then, T cells are re-engineered in a laboratory. The T cells are sent to a laboratory where they are genetically modified using non-virus and virus-

mediated transfer of nucleic acids into cells. The expression of foreign DNA produces CARs on the surface of the T cell. The CARs allow the T cells to recognize specific antigens on targeted malignant B-cells.[22,23] Next, the modified cells are grown in culture in the laboratory. This process can take a few weeks. Once a sufficient amount of CAR T cells have been expressed/grown, they are frozen and sent to the hospital where the patient is being treated.[22,23]

Prior to CAR T cell infusion, patients receive a brief course of lymphodepleting chemotherapy- referred to as conditioning therapy, to suppress the patient's immune system to prepare for incoming CAR T cell and to promote proliferation.[22,23] Next, the CAR T cells are thawed and infused into the patient's bloodstream in a process similar to a blood transfusion. These cells are then able to induce apoptotic and necrotic effects on the cancer cells that possess the targeted antigen on their surface. During this stage, patients may receive medication to prevent and/or regulate side effects (see Mitigating Side Effects section for more detail).[22,23] Lastly, patients who have received CAR T therapy have a risk/recovery period of two to three months, during which time they are closely monitored. CAR T cells may eradicate all cancer cells, however some may remain active in the body months after infusion.[22,23]

## CD19 Target

One of the most investigated targets for CAR-based therapy is the CD19 antigen due to its expression in most B cell leukemias and lymphomas, but absence in cells not in the B-cell lineage.[17] The CD19 antigen ultimately triggers cell lysis, therefore targeting this antigen aims to control tumour proliferation.[17] Furthermore, anti-CD19 CAR T cells have demonstrated successful results in the treatment of relapsed/refractory (R/R) B-cell malignancies, such as Non-Hodgkin lymphoma (NHL), ALL, and Chronic Lymphocytic Leukemia (CLL).[17] Clinical trials have been conducted since the initial success of CD19 CAR T therapy and have shown complete remission in 70-94% of patients with B-cell malignancies.[17] Promising outcomes in CAR T have been shown with the targets CD19, CD20, and CD30, expressing a CD28 or 4-1BB costimulatory domain, however the most success in clinical trials has been demonstrated by targeting CD19 for B-ALL specifically.[17]

The Food and Drug Administration (FDA) and other regulatory agencies in the pharmaceutical industry have recognized CAR T therapy as a revolutionary treatment and have approved two anti-CD19 CAR T

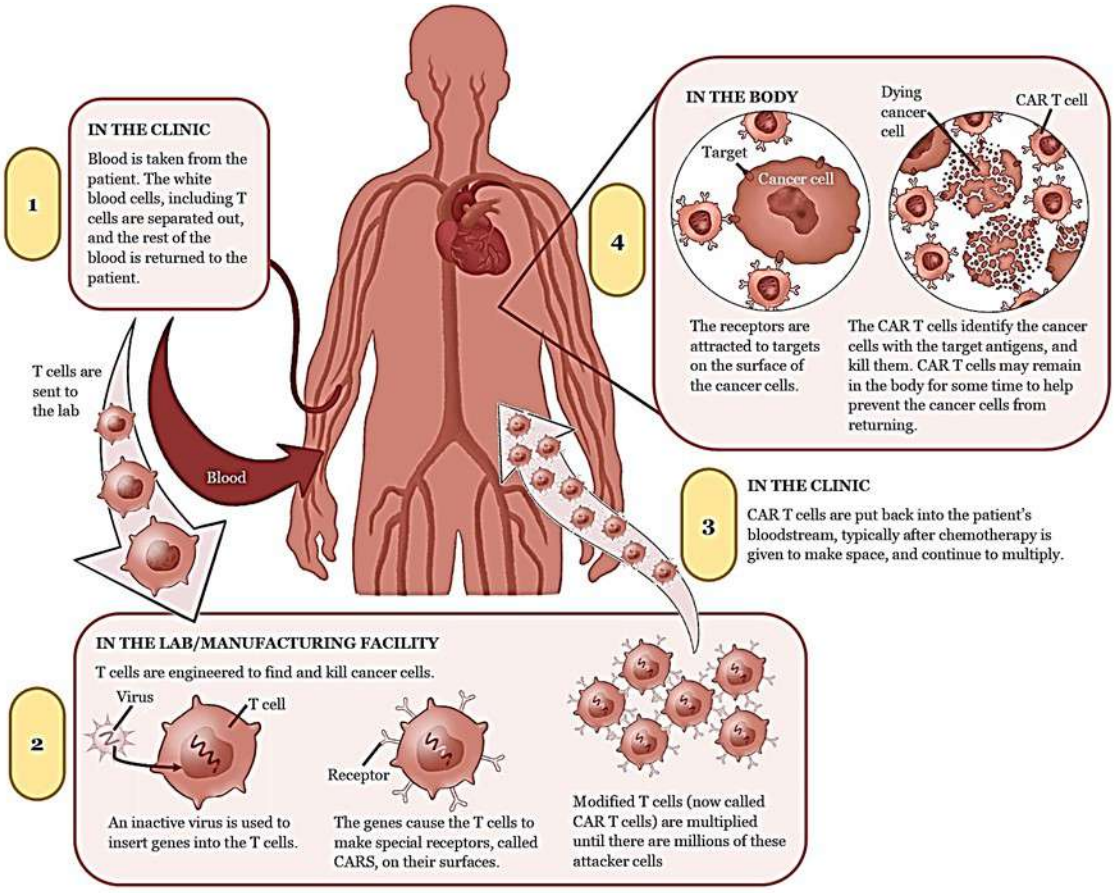


**Figure 2.** Visual representation of the multi-step CAR T cell immunotherapy procedure for infusing the genetically modified T cells in the patient's bloodstream to attack anti-CAR expressing malignant cells. Image adapted from (22).

cell therapies.[18,24] Tisagenlecleucel, also known as Kymriah, was the first CAR T therapy approved by the FDA on August 30, 2017, for the treatment of relapsed and refractory B-cell ALL and refractory diffuse large B-cell Lymphoma (DLBCL).[18,24] Axicabtagene ciloleucel, also known as Yescarta, is the second CD19-directed genetically modified T cell therapy approved by the FDA. It was approved on October 18, 2017, for the treatment of relapsed and refractory B-cell lymphomas and leukemias in adult patients.[18,24]

Each of the FDA approved anti-CD19 CARs contain either the CD28 or 4-1BB costimulatory domain interacting with the CD3-ζ chain.[19] Each of these domains induce a signal cascade that ultimately results in T cell activation, proliferation and necrotic/apoptotic effects on malignant cells.[19]

## MECHANISMS OF ACTION

### *CD3 Zeta Chain*

The multi-subunit TCR is composed of a TCR heterodimer, a ζ family homo-and heterodimer, and CD3 chains.[25] The CD3 chain is a protein encoded by the CD247 gene in humans and expressed on the T-lymphocyte surface. Activated TCRs induce tyrosine phosphorylation of the antigen recognition activation motif (ARAM) in the cytoplasmic domains of ζ chains and each of the CD3 chains.[25] After TCR activation, the cytoplasmic protein tyrosine kinase (PTK) ZAP-70 rapidly associates with the ζ and CD3 chains and undergoes tyrosine phosphorylation. The TCR-CD3 complex plays a key role in antigen recognition and transmembrane signalling.[26]

### *CD28 Co-Stimulation*

CD28 increases TCR sensitivity by lowering the signalling threshold required for T cell activation.[19] CD28 contains a cytoplasmic tail composed of several motifs that initiates specific protein-protein interactions.[20] Alternative functions of the CD28 domain, specifically in the immunological synapse between T cells and CD19 antigen presenting cells (APCs), includes cytokine production, cell cycle progression, apoptosis, epigenetic structure modification and metabolism.[19] The activation of CD28 requires binding with ligands CD80 or CD86 expressed on APCs.[19,20] However, it is suggested that CD86 plays a greater role in initiation and CD80 in maintenance of immune responses.[19] CD28 co-stimulation is critical for IL-2 secretion and Bcl-$X_L$ expression by the recruitment of the P13K/Akt pathway that has been previously activated by a series of signalling proteins. Bcl-$X_L$ is an anti-apoptotic protein that enhances cell survival whereas, IL-2 is a T cell cytokine necessary for proliferation.[20,27]

### *4-1BB Co-Stimulation*

4-1BB is a transmembrane protein expressed on activated T cells and APCs 4-1BBL is expressed on APCs which binds to 4-1BB to induce T cell responses via the tumour TNF-associated factors, TRAF 1 and 2.[19] This results in the subsequent activation of NF-kB, AKT, p38 MAPK and ERK pathways.[28] Through the previously mentioned pathways, 4-1BB signalling enhances T cell proliferation, cell cycle progression, cytokine secretion, cytolytic potential and resistance to transforming growth factor suppression.[19] This co-stimulation-mediated protein increases IFN and IL-2 secretion by $CD8^+$ T cells via T-helper cells (Th4), and IL-2 and IL-4 secretion by $CD4^+$ T cells.[19,29] IL-2 is a critical component for the growth and death factor of antigen activated T lymphocytes.[19,29] Alternatively, IL-4 serves as autocrine growth and differentiation factors, resulting in the proliferation and differentiation of T cells into effector cells.[30]

## CLINICAL TRIALS

Pre clinical trials of CAR T began in 2002 which provided early indications of CAR T efficacy.[31,32]

Table 1 outlines the stages of clinical trials for both Yescarta and Kymriah. The trials for Yescarta are currently active for ZUMA-2,3 and 4, however the therapy was approved by the FDA on October 18, 2017, based on early results from the ZUMA-1 trial.[33] Kymriah was approved on May 1, 2018, based on the JULIET trial, however, the ELIANA trial was underway during the approval process as well.[34]

The FDA approval of Yescarta and Kymriah CAR T cell therapy for the treatment of R/R B-cell malignancies was dependent on the results of the final stages of the clinical trials (Table 1). Based on the results of the ZUMA-1 trials for Yescarta, 83 % of the 108 CAR T cell infused patients indicated a degree of complete remission, with no deaths induced by the immunotherapy.[36,38] The following phases of the ZUMA trial (2 to 4) could not be analyzed since these trials are currently underway.

On the other hand, Kymriah FDA approval was attributed to the JULIET and ELIANA trials. The JULIET clinical trial results showed complete remission in 95% of patients after 3 months of CAR T cell infusion.[43] The death of three patients in the clinical trial was not attributed to Kymriah administration, or Cytokine-Release Syndrome (CRS) and neurological events caused by the therapy.[43] Additionally, the ELIANA trials demonstrated complete remission in 60% of infused patients. The high rates of remission in the trials of Yescarta and Kymriah have demonstrated the effectiveness of anti-CD19 CAR T cells against B cell malignancies in pediatric and adult patients.[43] The re-

sults of these clinical trials reveal the dramatic efficacy of CD-19-targeted CAR T cells which have induced complete remission in up to 90% of patients with relapsed/refractory B-ALL, that would have otherwise had an expected response rate of 30% with chemotherapy.[46] However, this success is accompanied by adverse effects, including cytokine release syndrome and neurotoxicity.[43] Post marketing studies have been conducted to assess long term safety and risks of secondary malignancies. Numerous studies continue to explore new ways of minimizing the side effects accompanying CAR T cell therapy. With the potential to mitigate these side effects, the applications of CAR T therapy for the treatment of alternative forms of cancer is endless.

**Table 1.** Summary of clinical trials leading to the FDA approval of Yescarta and Kymriah.

| FDA Approved CAR T Therapy | Clinical Trial | Patient Criteria | Implications of the Study | Results of the Clinical Trial |
|---|---|---|---|---|
| Yescarta | ZUMA- 1 | Involved 108 patients 18 years or older, with relapsed/refractory (R/R) large B-cell lymphoma.[35] All patients had previously undergone anti-CD20 monoclonal antibody treatment and an anthracycline-containing chemotherapy.[35] | This trial took place in 22 academic centers administering lymphodepleting chemotherapy drugs cyclophosphamide and fludarabine at least 3 days prior to the administration of an infusion dose of 2 x $10^6$ viable CAR T cells/ kg body weight.[35] | Two treatment related deaths occurred during the initial treatment but no deaths were reported after.[36] Grade >3 serious adverse events were reported in 48% of patients, with 11% experiencing severe cytokine release syndrome (CRS).[36,39] Follow up data collected from all 108 patients found that 83% had an objective response after 27 months indicating a degree of disease regression.[39] |
| | ZUMA-2 | Involved 130 participants with R/R mantle cell lymphoma who were 18 years or older.[37] Patients must have been previously treated with anthracycline or bendamustine-containing chemotherapy, anti-CD20 monoclonal antibody therapy as well as Ibrutinib.[37] | This trial took place in 19 academic centres, administering lymphodepleting chemotherapy drugs cyclophosphamide and fludarabine at least 3 days prior to administration of a single infusion dose of 2 x $10^6$ viable CAR T cells/ kg body weight.[37,38] | Results are not currently available as the trial is ongoing.[37] |
| | ZUMA-3 | Involved 100 participants with R/R B-ALL over the age of 18. Patients were previously treated with blinatumomab, CD19 tumour expression in bone marrow or peripheral blood.[40] | Administration of fludarabine and cyclophosphamide provided prior to intravenous injection of 2 x $10^6$ cells/kg body weight or 1 x $10^6$ cell/kg body weight.[40] | Results are not currently available as the trial is ongoing.[37] |
| | ZUMA- 4 | Involved 100 participants with R/R B-ALL between the ages of 2 and 21.[41,42] Patients must have previously been treated with 2 or more lines of chemotherapy or have refractory disease after stem cell transplant (SCT).[41,42] | Administration of fludarabine and cyclophosphamide provided prior to intravenous injection of 2 x $10^6$ cells/kg body weight or 1 x $10^6$ cell/kg body weight.[41] | Results are not currently available as the trial is ongoing.[38] |
| Kymriah (CTL019) | JULIET | Involved 99 patients, 18 years or older, with R/R diffuse large B-cell lymphoma (DLBCL).[43] The median number of prior lines of antineoplastic therapy was 3; 47% of patients had prior auto SCT. The median age was 56 years, 77% of those patients diagnosed with stage III or IV disease at study entry.[43] | This trial took place at 27 study centers in 10 countries on 4 continents .[43] Prior to infusion, patients underwent restaging and 93% received lymphodepleting chemotherapy.[43] | 86% of patients had grade 3 or 4 adverse events (AEs); CRS occurred in 58% of infused patients.[43] Three patients died within 30 days of infusion, due to disease progression.[43] No deaths were attributed to CTL019, CRS or neurological events.[43] CTL019 produced high response rates with 95% of complete remissions (CRs) at 3 months being sustained at 6 months in a cohort of highly pretreated adult patients.[43] |
| | ELIANA | Single-arm, open-label trial (all subjects are informed of study components) enrolled 92 patients, of which 75 patients were infused between the age of 3 and 23 years with R/R B-cell ALL. 8% of the participants had primary refractory disease.[44] All patients received a median of 3 prior lines of therapy: 53% received a SCT, and 8% received 2 SCTs.[44] | This trial took place in 25 centers in 11 countries.[44] | After a 13.1 month follow up, the overall remission rate was 81%. 60% of patients achieved CR and 21% of patients achieved complete remission with incomplete blood count recovery.[44,45] All infused patients with best overall response of complete remission were minimal residual disease (MRD) negative, 95% by day 28. 77% of patients experienced grade 3 or grade 4 CRS. In this trial 25 deaths were reported after CAR-T infusion.[44,45] |

## MITIGATING INHERENT SIDE EFFECTS

The most common side effects of CAR T therapy across many clinical trials is Cytokine-Release Syndrome (CRS) and neurological toxicities. CRS is a systemic inflammatory response triggered by elevated cytokine release, known as a cytokine storm, as well as increased T cell activation and proliferation, shown in Figure 3.[45] Currently, there are multiple grading systems to identify and clinically treat various stages of CRS. Davila et al. (2014) suggests using IL-6 protein levels as an analogous measurement of antibody levels, where an increase in either protein concentration is an indication of an inflammatory immune response.[47] Implementing a standardized monitoring system across all international treatment centres could help to optimize treatment methodology and improve the quality of life of patients.[47] Neurological toxicity is generally viewed as a secondary outcome to CRS although the mechanism by which this occurs is not understood.[48,49] Burdno et al. (2016) hypothesized that as IL-6 levels increase, this protein serum congregates in the brain and causes secondary toxicity effects.[48] Furthermore, a second hypothesis suggested by Rooney et al. (2018) includes IL-6 as a cause of CRS, but demonstrates IL-1 to be the cytokine that specifically causes neurological toxicity (Figure 3).[50]

In a foundational study conducted by Maude et al. (2014), 100% of patients treated with CAR T CD19 therapy experienced CRS symptoms, 27% of which experienced severe symptoms.[51] Symptoms of mild CRS commonly include fever, hypotension, rapid heart rate as a result of hypoxia, organ dysfunction, and rashing.[51] Mild to moderate CRS generally occurs within 4 days of the first infusion and is usually monitored for the length of the entire study.[53] Severe and life-threatening CRS symptoms reported in Yescarta clinical studies included cardiac arrhythmias, renal insufficiency, cardiac arrest or failure, capillary leak syndrome and macrophage activation syndrome.[52]

The immunosuppressant, Tocilizumab, is FDA approved for the treatment of severe CRS due to its function as an IL-6 receptor antagonist.[51] For both FDA approved CAR-T immunotherapies, Kymriah and Yescarta cite CRS as the most common side effect and recommend tocilizumab as a first response to any serious CRS symptoms.[48,52]

Neurological toxicities often occur concurrently to CRS, causing mild to severe toxicity symptoms. Mild forms include dizziness, confusion and delayed verbal response, which generally manifest within the first 4 days after infusion and subside within 8 weeks of CAR T therapy initiation.[54,55] More severe side effects include global encephalopathy, hallucinations, delirium, cognitive defects, seizures and cerebral edema.[52] A study conducted by Burdno et al. (2016) hypothesized that Tocilizumab cannot cross the blood-brain barrier due to its large molecular size.[48] As a result, corticosteroids are generally administered as a first line response to symptoms of neurological toxicity because the molecular structure and charge allows it to permeate the blood-brain barrier.[48,49]

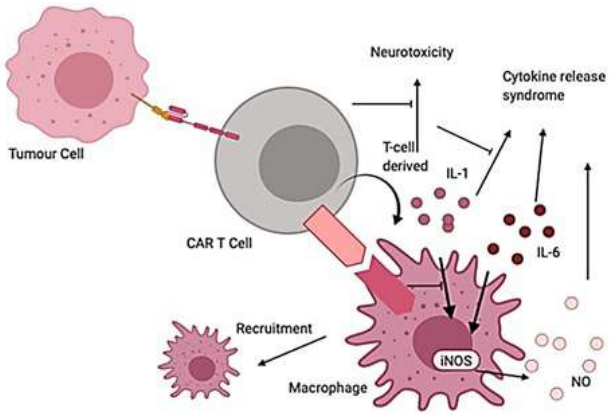


**Figure 3.** A simple model for CRS depicting the interaction between CAR T cells with tumour cells as well as macrophages. The macrophage interaction activates the release of IL-6 and IL-1 chemokines, which further activates the innate immune response and exacerbates the inflammatory signals causing CRS and neurotoxicity. Image adapted on BioRender.[53]

## FUTURE CONSIDERATIONS—VIABILITY FOR SOLID TUMOURS

### *Overcoming Microenvironment and Immune Suppression*

The microenvironment created by solid tumours presents many challenges for immune modulating therapies. One way to mitigate this challenge is by the introduction of a fourth generation CAR T cell that is "armoured".[56] These cells are engineered to withstand the toxic microenvironment by coupling the antitumour effect of CAR T with the secretion of IL-2 or IL-12.[56] IL- 12 is a heterodimeric cytokine produced by the activation of inflammatory cells such as T lymphocytes and natural killer (NK) cells.[57] To prevent toxicity associated with constitutive IL-12 production, a study by Zhang et al. (2011) attempted to create an inducible promoter for transcribing the protein.[57] To do so, an inducible retroviral vector was developed alongside a nuclear factor of activated T cells (NFAT)-responsive promoter to restrict IL-12 expression to specific tumour antigen recognition. Although this is the first study that has attempted to control IL-12 expression in this manner, the use of this method demonstrates promising results and is thereby continuing to be explored.[57] This modification is the beginning for establishing an "on and off" mechanism for future immune modulating therapies.[58]

A fundamental limitation to immunotherapies for solid-tumour cancers is the lack of adaptation to heterogeneity at the immunological level of the tumour lesion.[59] Antigen specific targeting provides powerful and long lasting effects against tumour cells, however, only on a very small scope of cells at a given time.[59] One way to navigate the restrictive nature of this therapy is to potentiate the endogenous immune system, suppressed by the tumour, to recognize and destroy cells not directly targeted by the CAR-T therapy.[57,59] IL -12 secretion can recruit and reinforce innate macrophage function, thereby encouraging the detection and destruction of antigen lacking cancer cells that would not have been otherwise detected by the therapy itself.[57,59] This accumulation of macrophages also contributed to a sustained antitumor response.[57] Thus, the induction of IL-12 provides access to otherwise inaccessible tumour regions as these cells are undetectable using the antigen targeting method of CAR T and provide a proinflammatory response.[58] Antigen-lacking cells are partially responsible for the progression of tumours despite treatment with antigen specific therapies, as they are able to evade the modified T cells and continue to proliferate and increase the strength of the tumour and the microenvironment it creates.[60] Since this proposed process would occur with the patient's innate immune system, the toxicity associated with this IL-12 catalyzed process is theoretically far less than direct CAR T binding and subsequent cytokine release.[59] More research is required to find other potential immunostimulatory cytokines to create an immunological memory and independently suppress the reoccurrence of tumours using the innate immune response as a first line of defense.[60]

### *Overcoming Physical Barriers*

There are also physical barriers that must be mitigated. Specifically, when treating solid tumours present in the epithelial and mesenchymal compartments which are not significant in hematopoietic cancers.[55] One study by Pegram et al. (2014) has shown that engineered T cells may not currently be equipped with the tools required to break through these outer barriers compared to natural immune cells.[60] The trafficking of immune cells towards the tumour foci is significantly inhibited by the overexpression of ligands and receptors on the tumour endothelium.[60] T lymphocytes are able to degrade the extracellular matrix specifically the heparan sulphate proteoglycans (HSPGs) component during extravasation.[55] Natural T cells do this by expressing heparanase (HPSE) to degrade the HSPGs and access the tumour cells.[55] Recent studies show that HPSE mRNA is downregulated in *in vitro*-expanded T cells, therefore by engineering cells to express or overexpress this enzyme, CAR T cells may be able to infiltrate further into stroma-rich solid tumours and decrease the barriers associated with solid tumour contact.[55]

## BUDGET PLAN AND TREATMENT OPTIONS

Although CAR T therapy has shown promising results for the treatment of R/R B-ALL, it is critical to note the accessibility of this new innovative immunotherapy to the public. In order to determine the most beneficial treatment plan for a patient suffering from ALL, it is important to consider all treatment options, while concurrently observing the financial burden each treatment would impose on the individual.

Currently, the cost of CAR T therapy in the United States ranges from $373,000 to $475,000 for Yescarta and Kymriah, respectively.[61] The high estimated cost of these therapies does not include hospital stays and extended treatments for patients who experience CRS or other adverse effects. It is important to note that there is no information available for CAR T therapy in Canada as it has only been conditionally approved by Health Canada since September 5, 2018. However, it is not available to Canadian residents yet as several components of this decision need to be finalized. Currently, Health Canada offers coverage for patients 3-25 years of age.[61] This however does not specify coverage for leukapheresis, or other essential techniques associated with the therapy. Negotiations are ongoing between Novartis and Canadian Cancer Care Ontario, with plans to reduce the manufacturer cost by at least half before government coverage is offered to the public.[62] Using the assumption that the procedures associated with the CAR T treatment are not covered under this negation, the cost should be minimal. For example, the leukapheresis procedure necessary to extract the white blood cells will cost approximately $550 CDN.[63] This estimate is based on the current rate of plasma exchange procedures which is another type of apheresis treatment that requires similar equipment and treatment costs. Currently, Kymriah's only trial centre in Canada is located at the SickKids hospital in Toronto, therefore creating an additional cost for those that live out of province.[64]

Based on the cost analysis of the current treatment options, there are many financial implications that accompany these treatments for patients with B-ALL, apart from the therapy itself.[65] These include hospital stays, therapy cost, ongoing physician care and transportation costs if the treatment is only offered at specific institutions.[66,67] The standardized treatment options for B-ALL are chemotherapy, radiation therapy, or stem cell transplants.[67] Of these three options, chemotherapy and radiation therapy require multiple visits by patients undergoing this particular treatment plan. Based on this, it is important to consider that the individual would experience higher hospital care and transportation costs, compared to the same patient undergoing a stem cell transplant or CAR T therapy. Stem cell transplants usually require patients to remain in the hospital for a period of 2-6 weeks until the

patient's blood cell count returns back to normal levels after transplantation.[67,68] In contrast, CAR T therapy patients usually stay in the hospital for at least 7 days after receiving treatment, and are required to stay within 2 hours travel time of the hospital for regular follow ups until at least 4 weeks after leaving the hospital.[69] The cost to stay in a location within this transportation time range is an additional cost to consider, however, the Canadian Cancer Society offers cancer lodges that are available to cancer patients with a lower price for overnight stay, including meals.[70] It is important to note that chemotherapy treatment can be 4-6 months in length.[68] With this treatment option in mind, transportation, extended stay near the treatment centre, and other additional costs which can extend over a relatively long period of time can result in accumulating financial burden. In addition to the primary cost of all treatment modalities, secondary costs associated with hospital stay must be accounted for when analyzing treatment options. For example, radiation therapy is administered in fractions lasting approximately 5-8 weeks, with the number of fractions depending on the treatment plan.[71] Although this is an outpatient service, daily radiation treatments require additional costs similar to those for chemotherapy and stem cell transplant.[36]

The present cost of CAR T therapy is out of reach for those without coverage or adequate personal funding. Therefore, it is not recommended for patients who have access to less expensive options. Due to the high remission rate demonstrated by clinical trials, CAR T is an effective option for patients who have not seen success with the traditional treatments, in which case CAR T would be recommended based solely on successful remission rate. Overall, CAR T therapy is extremely effective, and is recommended for those who can afford it at its current price. Every cancer treatment has the potential to be a large financial burden on the patient and their family, therefore it is necessary to plan for each unique case. CAR T therapy has promising advancements in the future as a cancer treatment and could one day become a main treatment modality.

## CONCLUSION

B-ALL is an aggressive leukemia subtype with wide genetic variation requiring individualized treatment. Current treatment options include general systemic chemotherapy and combined therapeutic treatments. By utilizing the body's immune system, a greater level of personalized medicine can be achieved as the procedure of CAR T therapy retrieves and modifies T cells directly from the patient. The CD19 target in particular has shown great promise since it is only expressed on malignant B cells in B-ALL. Additionally, both of the current FDA approved treatments; Kymriah and Yescarta utilize the CD19 target. Several clinical trials including those for Kymriah and Yescarta have shown an improved safety profile and remission rate. The side effects caused by these therapies can be severe such as neurotoxicity and CRS, however there are now many techniques to mitigate adverse effects to ensure patient safety. The next step for CAR T therapy will be to overcome the physical and physiological barriers associated with solid tumours, in order to become a viable treatment option for solid tumour cancers in the future. CD19-targeted CARs have paved the way for engineered T cell therapies with high response rates against relapsed and refractory B-cell malignancies. Although CAR T has shown beneficial responses in highly refractory populations, there are several limitations that must be considered.[71,72] The complex preparation, economic factors, lack of accessibility in certain countries (ie. Canada), and the inability to successfully treat solid tumours complicates the potential for this therapy to reach a broader public. As CAR T cell technology continues to develop, there is the potential for the discovery of a more viable, affordable CAR T treatment option with improved safety and efficacy.

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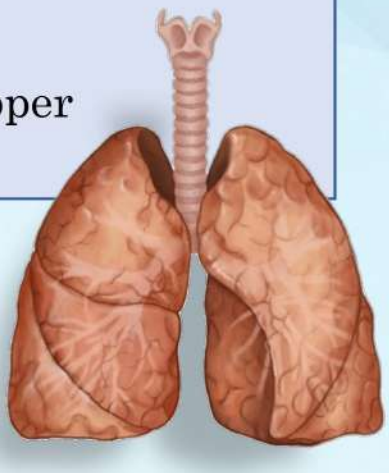
# COVID-19

A new strain of coronavirus known as COVID-19 which stands for Coronavirus Disease 2019 was discovered in December 2019 and initiated in Wuhan, Hubei province, China.[1] The virus has infected over 300,000 individuals globally as of March 2020.[2]

Although little is known about the new strain, it is the seventh known coronavirus to infect humans.[3]

Out of the first 44,672 confirmed cases in Wuhan, China 86.6% were between the age of 30-79 years.[4]

Infected individuals commonly experience upper respiratory tract infections.[3]



The number of deaths has surpassed 4,000 and the World Health Organization has labelled the virus as a global health emergency due to the high infectivity of COVID-19.[2]

A recent report from Germany shows that asymptomatic individuals who are infected with COVID-19 are infectious and can spread the virus.[5] The best way to prevent the spread of viruses is to practice good hygiene including regular hand washing and avoiding contact with others when sick.

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Due to the global response towards COVID-19, new technologies are being explored to detect the virus in individuals. Most recently, a form of real-time reverse-transcription PCR was developed – a rapid and robust diagnostic tool that helps identify whether mRNA specific to COVID-19 is expressed in an individual. A positive test for viral mRNA validates whether an individual is infected.[6]

Due to early detection of the COVID-19, the genomic sequence of the virus has been identified and helps detect those who may be infected. Yet, the virus continues to spread to new countries due to international travel.[7]

The current antiviral drugs known as remdesivir and chloroquine have been shown to be effective in controlling COVID-19 infection *in vitro*, but clinical studies must be conducted to determine its efficacy in humans.[8]

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# INTERVIEW

## WITH DR. TOBIAS BERG

# BATTLING ACUTE MYELOID LEUKEMIA (AML) AS BOTH A CLINICIAN AND SCIENTIST

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## ABSTRACT

Acute myeloid leukemia (AML) is characterized by the accumulation of immature hematopoietic cells in the bone marrow that impair normal blood formation. Chemotherapy is always the first treatment option for AML. Patients can also be cured by allogeneic stem cell transplantation, which consists of transferring stem cells from a healthy donor to the patient after high-intensity (high-dose) chemotherapy. Many mutations found in AML affect the cells on an epigenetic level, influencing the gene expression of the cells such as influencing DNA-methylation genes and chromatin-modifying genes. Affecting these epigenetic mechanisms is therefore of great interest in the area of AML.

**Dr. Tobias Berg,** MD, Ph.D.

*Dr. Tobias Berg is a clinician-scientist who was recently appointed the Boris Family Chair in Leukemia and Hematopoietic Stem Cell Translational Research in June 2019. Prior to joining McMaster, he specialized in Hematology and Oncology at the University of Frankfurt with a focus on epigenetics, AML, and allogeneic stem cell transplantation. After completing his doctoral project at the University of Minnesota and residency at the University of Freiburg, Dr. Berg completed a postdoctoral fellowship at the B.C. Cancer Agency. When asked about his overall experience at McMaster he stated that, "My experience here at McMaster so far has been very good. I have a clinical practice at the Juravinski Cancer Centre and have my research work at the university. I've been welcomed very warmly, and everyone was very supportive, it's great. There are certainly challenges when being a new faculty member, and they've been very helpful when starting up the lab."*

**► Why did you choose to work in the field of Leukemia and Hematopoietic stem cells?**

So, I was fascinated by the field of hematology, generally in the field of cancer, since high school. During the early phases of my medical studies, I got more and more interested in cancer as this is probably the area in medicine where our growing understanding of underlying biological processes of disease is probably leading to the greatest improvements. Also, during my doctoral thesis, I developed a growing interest in immune tolerance which brought me in contact with the

field of allogeneic stem cell transplantation.

**► Your research focus has been on adult AML and providing novel treatments. What is your approach in developing some novel treatment options?**

AML treatments for a long time have been more or less the same. The treatments that are still used as the backbone of our treatments were first published before I was born! This standard regimen that we are using is called 7+3. It is seven days of cytarabine and three days of daunorubicin. That, as well as allogeneic stem cell transplantation, was developed in the early 70s. So, there was a very long gap period in which there were not many new developments. We are now in a time where we have a lot of new approval of drugs in the area of AML. When the patient is diagnosed with leukemia, the immune system has already lost the fight. So, we either have to get the immune system back on track or give them another immune system using allotransplantation. And then the epigenetic treatments can help the new immune system to fight cancer by essentially getting genes expressed that would otherwise not be expressed. I am very excited about this development and about integrating these into treatment algorithms. I am also very interested in contributing to the development of novel treatments by understanding the epigenetics of AML and interactions between genetic mutations and epigenetic regulation.

**► You also work on epigenetic regulators and their interplay with genetic regulators. What is your stance on the role of genetic vs environmental factors in cancer development?**

In general, both play a role. There are genetic syndromes in the area of AML that make a patient prone to the development of AML, but it is rare. And, there are obviously environmental factors that enhance the risk of acquiring spontaneous types of mutations. So, they are both important.

While I often get the question from patients about the direct cause of their disease this is often very hard to say, because mutations often arise spontaneously in rapidly dividing cells. With a bit of bad luck these mutations occur in genes that are important for the growth regulation or the differentiation of cells which can be the first step in the development of cancer.

"The treatments that are still used as the backbone of our treatments were first published before I was born!"

**► What are some challenges in developing treatment options for AML?**

There are two main challenges in the area of AML. Number one is treatment resistance. We have chemotherapy regimens that 60-80% of young patients respond to, while for old patients, the rate is less, maybe 40-60%. And then some patients cannot tolerate such intensive treatments. So, treatment resistance is a very important area. Number two is relapse. We need to understand what leads to the relapse of leukemia. Both treatment resistance and relapse are interconnected because if you cannot eradicate the leukemia cells, then the residual cells are the source of the relapse. This connection is a challenge and also an opportunity. Our group is therefore very interested in understanding the biological processes in these residual cells after treatment.

"Both treatment resistance and relapse are interconnected...if you cannot eradicate the leukemia cells, then the residual cells are the source of the relapse."

**► What is your advice for students and researchers who want to pursue the field of stem cell research?**

It is a very active area of research. Stem cell research can be connected to several things such as mechanisms in cancer and leukemia. If you want to get involved in that type of research, a strong molecular biology and biochemistry background is helpful. Also, a good background in the area of bioinformatics is helpful because a lot of studies in this area, particularly genetic studies such as single-cell sequencing, require the use of advanced analysis tools to analyze large data sets. Stem cell research is also very interdisciplinary. For someone who is more interested in stem cell research from a medical perspective, it is where people from various fields of medicine and research come together, and interdisciplinary communication is the important thing which will drive this field. So, whatever you are interested in, either more on the research side or more on the clinical side, it is essential to understand this interface and connection.

# Could a Methyl Group Predict Your Risk of Depression?


## ARTICLE INFORMATION

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## ABSTRACT

Major Depressive Disorder (MDD) is a systemic condition that diminishes the daily quality of life of those affected. There are no current methods that can reliably diagnose depression on a biochemical level. The premise of this work is to report on a potential biochemical marker, DNA methylation of the serotonin transporter gene (5-HTT). This biochemical marker can serve as an indicator of gene expression patterns, ultimately leading to a neurochemical imbalance in affected individuals. Studying this biomarker has the potential to improve diagnostic and therapeutic techniques in the future, and improve the prognosis of those with MDD.


**Keywords:** Major depressive disorder, serotonin, DNA methylation, biomarker

Major Depressive Disorder (MDD), or clinical depression, is one of the top ten global health burdens. The highest prevalence of the disorder is observed in the older-adult cohort, which are adults between the ages of 40 and 64.[1] As characterized by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), depression is diagnosed upon experiencing a culmination of a minimum of five of the following criteria in the continuous duration of a two-week period: despondent mood, reduced satisfaction from and inclination to partake in daily activities, diminished appetite, fatigue, reduced physical activity, inapplicable or excessive feelings of guilt and worthlessness, decline in the ability to concentrate and reason, and the presence of suicidal thoughts or tendencies.[2] One of the first two characteristics must be present for the diagnosis to be established.[2] Depression is a multifactorial disorder, where the contributing factors stem from both genetic and environmental constituents.[3] The current understanding of the etiology and pathophysiology of MDD is inadequate, hindering opportunities for a positive prognosis, especially in the older adult population who experience a more chronic outlook for most disorders,[4] likely including MDD. Considering the scarcity of information pertaining to the root causes, chronicity, and its heterogeneous nature, genomic and epigenomic studies are conducted to investigate the biomarkers involved in acquiring MDD.

Epigenetics is the study of heritable modifications of gene expression that do not involve changes to the nucleotide sequence of the genome.[5] Thereby, this field investigates the biochemical mechanisms of action and the phenotypic consequences of epigenetic markers such as methylation, acetylation, and phosphorylation, on the activity of genes in different cell types. Methylation is the addition of a methyl substrate onto a nucleotide and typically represses gene transcription. Methylation occurs at cytosine residues, which are usually immediately followed by guanine nucleotides. Together, these monomers are called CpG islands.[6]

Previous studies on the neurobiology of depression have identified the deficit of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT), the brain's "happy" chemical, as the main biomarker of MDD.[7,8] Although, the simultaneous disturbance of multiple biochemical mechanisms is now widely considered to iteratively postulate the likelihood of acquiring the disorder,[3] research related to serotonin remains the current focal point in MDD etiology.

Serotonin is a chemical produced by the nerve cells and is involved in regulating the circadian rhythm, digestion, and cognitive functions.[7] Consequently, it impacts many biological processes, including those related to the etiology of MDD.[7] On a cellular level of neural communication, one neuron acts as a transducer, and the other acts as a recipient of a chemical signal.[9] When serotonin is released by the transducing neuron,

it is localized in the synapse prior to its uptake by the receiving cell.[9] In depression, the reduced amount of serotonin is due to the malfunction of the serotonin reuptake transporter (5-HTT), a protein that recycles the neurotransmitter before it arrives at the receiving neuron.[10] Encoded by the serotonin transporter gene (5-HTT), its expression is regulated by an upstream repeat polymorphic promoter region (5-HTTLPR), which is generally shorter in individuals exhibiting depressive symptoms.[10] Additionally, another regulatory region has been linked to the modulated expression of 5-HTT, a CpG island located upstream of exon one and the transcriptional start site of the transporter gene.[10] DNA methylation of the CpG island has been associated with depression; however, mechanisms inducing this epigenetic modification are currently unknown.[10]

A study by Lam et al. (2018) investigated the genotypic variation of the 5-HTT gene as a contributing factor to DNA methylation and depression in the older-adult cohort. In all subjects, depression was diagnosed using either DSM-IV or a score above 16 on the Center for Epidemiologic Studies Depression Scale (CES-D), a self-report depression scale. Both diagnostic measures signify high levels of depressive symptoms.[10] A total of 302 individuals were recruited, and genomic DNA from white blood cells was sampled for analysis.[10] Recruits were a cohort of 95 depressed and 207 non-depressed participants.[10] Most individuals from the former group were underprivileged women with psychological and physical comorbidities.[10]

Two investigations were performed from collected genomic samples: genotyping and methylation pattern assay.[10] Genotyping is the process of identifying individual genetic differences in computational comparison to the reference sequence.[11] Most individuals in the study exhibited a homozygous S or L genotype, where 5-HTT alleles were either short or long, respectively.[10] Methylation patterns were assessed using sequencing post-bisulphite exposure, which is a treatment of the genomic sample that permits the identification of methylated regions. This allows for the simultaneous depiction of multiple CpG regions throughout the genome.[10] Statistical analysis was executed to determine univariant relationships between genotype and DNA methylation, and DNA methylation and depression status.[10] The analysis also accounted for confounding factors, such as a record of previous MDD, use of antidepressants, and documented comorbidities.[10] Upon multivariate analysis and the implementation of the necessary statistical adjustments, the study ensured validity in its evaluation of the genotype, DNA methylation, and depression associations.[10] Lam et al. (2018) identified a statistically significant relationship between genotype and DNA methylation prevalence. In individuals with homozygous S alleles, decreased methylation contributes to depression, whereas in those with homozygous L alleles, higher levels of this epigenetic mark are associated with depression.

Lam et al. (2018) have conducted a large-scale study with a diverse spectrum of participants, perpetuating a reliable extension of the findings to the general population. As a cross-sectional investigation, however, a major limitation is the lack of longitudinal data which would have been essential in understanding the patterns of DNA methylation that change with age. On the other hand, one must acknowledge the reversible nature of epigenetic modifications, and therefore, consider the colossal importance of the study of such mechanisms as a viable option for implementation in diagnostic and therapeutic regimens in the future. Additionally, in the study by Lam et al., (2018) DNA was collected from white blood cell (WBC) samples, discounting differential methylation patterns in other cell types, such as neural cell lines. However, depression is a systemic disorder that has the propensity to affect other organ systems. Given the infeasibility of sampling all the organ systems affected by these methylation patterns, WBCs are an appropriate source of genomic samples, as they are distributed ubiquitously throughout the body via the bloodstream.

To date, serotonin deficiency is one of the main working models as the cause of depression. Research by Lam et al. (2018) explored serotonin's role by using DNA methylation and the 5-HTT genotype as associated factors. In light of the discussed findings, the main implication of this research is the implementation of 5 -HTT DNA methylation as a biomarker for MDD.

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# A New Hope for Delaying Clinical Onset of Rheumatoid Arthritis: Early Intervention with Rituximab


## ARTICLE INFORMATION

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## ABSTRACT

Rheumatoid arthritis (RA) is a highly prevalent autoimmune disease that affects 16 million people globally. It is caused by an inflammatory autoimmune response that results in swelling of the joints and chronic pain. While we know that RA operates via the immune system, the specific mechanisms of RA pathogenesis are not fully understood, making diagnosis and treatment options limited. Rituximab, a monoclonal CD20 antibody, is a current form of RA treatment that specifically targets autoreactive B-cells to help mitigate the symptoms of RA at the clinical stage. Gerlag et al. (2019) outline a preventative window of opportunity for preclinical RA intervention with rituximab and identified two predictive biomarkers through exploratory methods. Their findings demonstrate that early administration of rituximab during preclinical RA delays disease onset and impedes its progression. This timeframe for intervention offers a promising first step for future studies investigating RA mechanisms and early treatments.


**Keywords:** Rheumatoid arthritis, rituximab, B-cell directed therapy, preclinical intervention


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Arthritis is currently a prevalent chronic health condition amongst Canadians, which results in reduced quality of life and loss of productivity.[1,2] Autoantibody positive rheumatoid arthritis (RA) is a common form of arthritis that affects over 16 million people worldwide.[2] As an autoimmune disorder, RA develops as a consequence of autoantibody formation and is characterized by joint inflammation, stiffness, and damage, which leads to systemic chronic pain.[2] Although individuals can be diagnosed and treatment options are available to reduce symptoms, there is currently no known cure due to uncertainty surrounding the biological mechanisms that underpin the disease.[3,4] Additionally, it is difficult to establish a concrete timeline for how RA develops since disease onset and progression varies between individuals due to its complex pathogenesis.[3,4] However, intervention ahead of RA onset could thwart the disease altogether, suggesting a preventative window of opportunity for treatment.[5] Evaluating the effects of B-cell therapy on preclinical RA, the stage before arthritic development, is an opportune origin for exploratory research.[5] The preclinical phase entails the presence of RA biomarkers prior to the clinical manifestation of the disease. This presents an opportunity for earlier diagnosis and treatment and may allow for earlier applications of preventative measures. Inflammation in RA is associated with an immunological response. This response is partially mediated by antigen-presenting B-cells, their cytokines, and their associated antibodies.[6] B-cells can serve as efficient antigen-presenting cells, which activate T-cell responses, thus resulting in inflammation. B-cells also produce cytokines that enhance inflammatory responses. The antibodies produced by B-cells also trigger complementary activation, which further promotes inflammation, making B-cell receptors a key target in early diagnosis and potential treatment for RA within B-cell therapy.[7]

The antibody, rituximab, is an important factor when attempting to prevent the development of RA. Originally used to treat lymphoma, rituximab is a drug used in B-cell therapy. Rituximab works by initially binding to the CD20 receptor on a B-cell, prompting the cascade of numerous systems, including apoptosis and cytotoxicity (Figure 1).[8] This is effective for arthritis treatment, especially in the preclinical stage, as the specificity of antibodies allows for specific binding to CD20+ autoreactive B-cells.[8] After binding to the cells, rituximab induces antibody-mediated cytotoxicity, which involves the binding of an effector cell to the rituximab-CD20 complex, resulting in the lysis of the autoimmune B-cell.[8,9] The variability of these systems promotes the clinical efficacy of rituximab, which assists with the creation of novel treatment regimens.[9] The high prevalence and disease burden of RA drives the need for additional research, specifically with regards to rituximab-induced B-cell therapy, to mitigate RA's progression and prevent its onset.

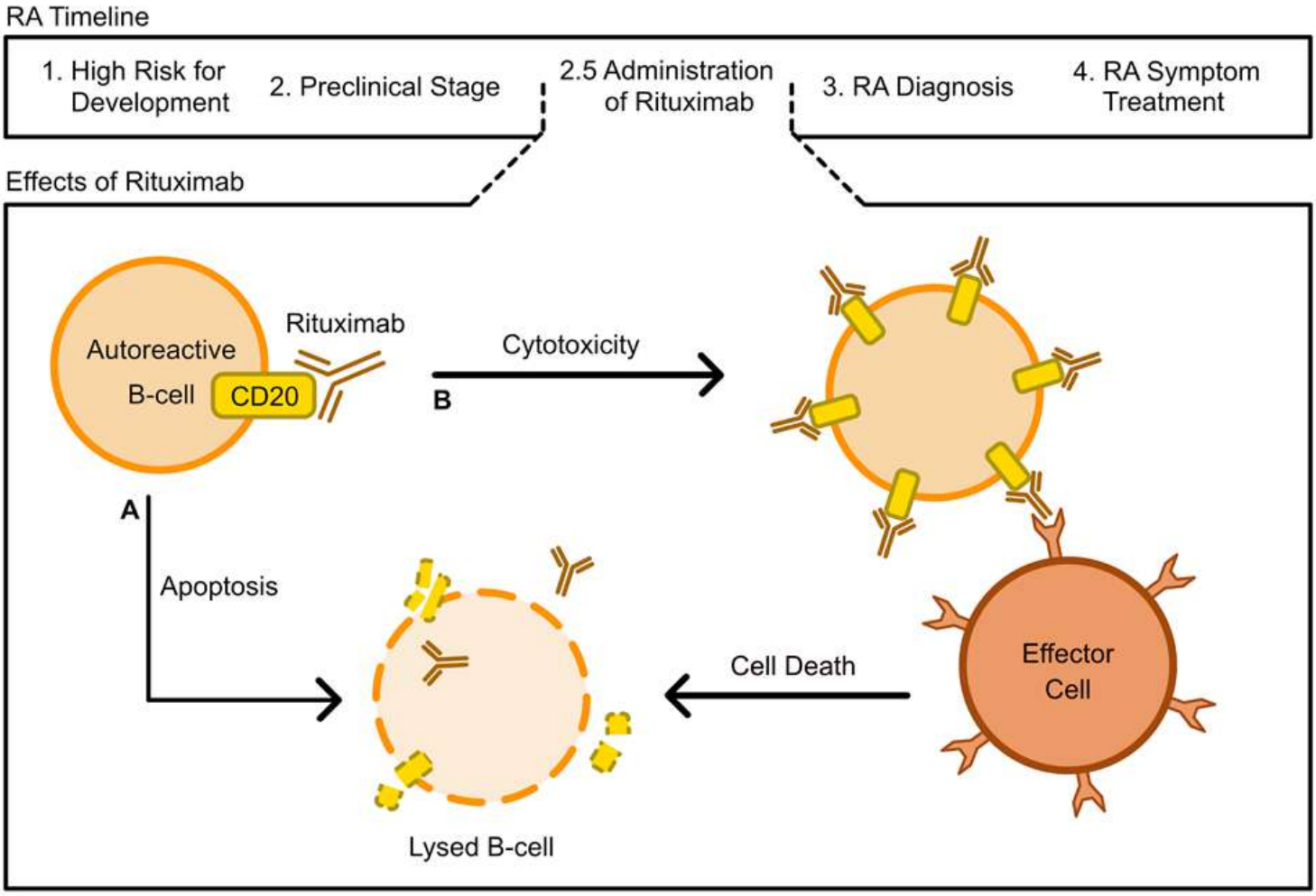


**Figure 1. Illustration demonstrating the effects of rituximab in rituximab-induced B-cell therapy.** Upon administration during the preventative window of opportunity, within the RA timeline, rituximab works to selectively target CD20 receptors on autoreactive B-cells. This results in either (**A**) apoptosis and death of the cell or (**B**) cytotoxicity. On pathway **B**, effector cells recognize rituximab and promote cell death, limiting the number of autoreactive cells in the body.[5,8,9]

A study by Gerlag et al. (2019) explored the effects of B-cell directed therapy using rituximab in individuals during the preclinical phase of RA.[5] 81 subjects were recruited based on the presence of biomarkers, such as rheumatoid factors (RF) and anti-citrullinated peptide antibodies (ACPA).[5] These risk factors indicate a 40% increase in the risk of RA development within two years.[5] Macrophages may be activated by RF and ACPA, resulting in increased cytokines and chemokines associated with the clinical manifestation of RA.[5] Of the 81 subjects, 41 received a single infusion of 1000 mg rituximab and 40 received a placebo.[5] Statistical analyses revealed a significant decrease in the number of B-cells within four weeks post-treatment, along with significant drops in multiple RFs.[5] Rituximab intervention reduced the risk of arthritis development by 55% at 12 months, and 53% at 18 months.[5] Although the effect of rituximab was statistically significant, its preventative effects decreased over time, showing temporary prevention.[5] Researchers suggest the clinical phase of RA can be avoided through repeated treatment with rituximab, supporting the idea of a preventative window of opportunity.[5]

Whether repeated treatment has this potential is an important research question with revolutionizing outcomes, but requires further investigation.

Through exploratory methods, the researchers also identified two biomarkers effective in predicting the onset of RA: erythrocyte sedimentation rate and anti-citrullinated $\alpha$-enolase.[5] Considering the preventative window of opportunity, the integration of these predictors into the overall B-cell treatment regimen is important in successfully implementing the intervention prior to pathogenesis. This is why future research focused on increasing our knowledge of reliable diagnostic predictors is necessary to provide more effective RA treatment. It is also worth noting that patient heterogeneity and the complexity of RA itself can interfere with successfully determining at-risk individuals prior to pathogenesis. Therefore, being limited to only two predictors is insufficient. This emphasizes the necessity to discover additional predictors to broaden the extent to which at-risk individuals are identified.

Current treatments are only administered in RA patients after the clinical onset of the disease, but it is now possible to identify risk factors during earlier stages before RA onset.[5] Gerlag et al. (2019) demonstrate a potential preventative window of opportunity where early intervention would delay the development of clinical RA and allow for greater chances of disease remission.[5] The preventative window outlined in this study describes the optimal time to target and eliminate autoreactive B-cells. These targets provide opportunities to predict mechanisms of attack and whether the current state of autoimmune B-cells in a patient's body lead to RA. Identifying the disease pathways via autoimmune B-cells can identify the immunological malfunctions that contribute to RA pathogenesis. Despite the promising conclusions from this study, the chosen sample solely being from the Netherlands may impact the generalizability of the results, as there is uncertainty regarding the reproducibility of the results in other populations.[5] Nevertheless, the findings serve as a gateway for other autoimmune-related disorders, providing the option to detect and identify autoimmune disorders prior to the emergence of signs and symptoms. Moreover, this new understanding of the clinical manifestation of the disease, through B-cell autoreactivity, provides further insight into potential treatment.

RA is a debilitating condition that impacts millions of people globally, reducing their quality of life while imposing a large financial burden. Intricate pathogenesis involving autoimmune B-cells contributes to the difficulties of diagnosing the disease, with current treatments only being administered after clinical onset. Gerlag et al. (2019) outlined a preventative window of opportunity and found two predictive biomarkers.[5] They demonstrated that rituximab intervention within the preclinical phase, during the preventative window, can temporarily prolong RA development. The findings are vital as they suggest that repeated administration of the treatment might be able to permanently delay RA's clinical development, although future studies that test this hypothesis are needed.[5] This could have monumental implications, preventing the onset of RA in future populations.

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OPINION PIECE

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# A Bioethical Analysis of Gene Editing

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## ABSTRACT

New developments in gene editing methods include the possibility to alter embryos for disease resistance. This could allow for increased immunity in the future, but at what cost? Gene editing may have unintended consequences. Some alterations may prevent the development of one disease but increase susceptibility to another. Other genes persist in populations for complex evolutionary reasons. Scientists must therefore consider the consequences and bioethics associated with these genetic changes. With examples such as the CCR5 coreceptor and major histocompatibility complex, it becomes clear that this type of genetic enhancement is immoral when evaluating it from biological, evolutionary, social, and economic perspectives. First, having the ability to select for certain desirable genes limits genetic diversity, which creates a barrier for evolution. Selecting for certain genes perpetuates the concept of *ideal genes* resembling dangerous eugenic ideologies. Should these procedures become more prevalent, the issue of accessibility arises. If these expensive procedures are only available to those who can afford them, the opportunity gap between the poor and the rich will widen. An investigation of case studies and ethical implications demonstrates that genomic editing is immoral and impermissible.

**Keywords**: Bioethics, gene editing, genomics, CCR5, MHC, evolution

In 2018, Chinese scientist He Jiankui shocked the world by stating that he had altered the genome of two embryos to confer resistance to the Human Immunodeficiency Virus (HIV).[1] This sparked debate in the scientific community and brought an ethical debate on the permissibility of gene editing to the forefront.[2] On one hand, alterations could provide immunity to genetic conditions. However, risks far outweigh potential benefits. The genome is complex, and protective alterations for one medical condition may pose a risk for another debilitating disorder. Additionally, many genes persist in the population for evolutionary purposes, and the removal of a malicious gene could have unintended consequences. For these reasons, attempts to edit human embryos are immoral.

One example of the ramifications of gene editing is He's embryonic alterations of the CCR5 coreceptor, which is a protein that allows HIV to enter human cells.[3] The literature shows that those who are homozygous for a 32 base pair deletion in the gene (CCR5-delta32) are resistant to HIV.[3] However, the homozygous mutation that confers HIV resistance increases susceptibility to flaviviruses like Zika and West Nile Virus.[4,5] Studies in mice without wild type CCR5 proved fatal upon infection with West Nile Virus.[6] Furthermore, in a meta-analysis of American cohorts, a higher number of patients with West Nile Virus are homozygous for CCR5-delta32 than those that possess the wild-type alleles.[7] Even more concerning, around 5% of West Nile patients had this homozygous genotype, which is higher than the 1% in a typical population.[6] While the promise of preventing HIV may seem tantalizing, scientists are exposing altered embryos to the possibility of developing other dangerous and potentially fatal diseases.

Many genes that seem detrimental in a population may be advantageous in other ways, which is why gene editing should be prohibited. The major histocompatibility complex (MHC) is a section of the genome that helps regulate the immune system, and human leukocyte antigen (HLA) genes in this area have many genetic variations.[8] This likely fosters pathogen resistance and makes the immune system adaptable. However, the MHC region also has genes that are linked to autoimmune conditions, cancers, and schizophrenia.[9] Since these detrimental genes are in close proximity to the HLA genes, they "hitchhike" with the beneficial HLA genes; they cannot be eliminated with-

out eliminating pathogen resistance.[9]

These examples demonstrate that gene editing should not be permitted . The genetic hitchhiking described above creates evolutionary trade-offs, as editing the genome could remove a detrimental gene at the expense of another. This also raises larger issues about which alterations should be pursued. This may reduce population diversity, which is a barrier to evolution. The effects of genetic selection may mimic populations affected by genetic drift, as certain traits may disappear with changes in the frequency of certain genes perceived as desirable or undesirable.[10] Although more desirable traits may seem to be genetically favourable, cases of genetic hitchhiking and the breaking news about He's experiment suggest the importance of these genetic tradeoffs. This artificial selection of traits may disrupt natural selective pressures. The decrease in genetic diversity may then make populations more susceptible to changes.[10,11] This evolutionary perspective is crucial in the ethical debate surrounding gene editing, and no physician should be given this power.

In addition to the complexities outlined, there are questions about what might happen when scientists cross the line between gene therapy for the treatment of genetic conditions and enhancement of already healthy genes. When scientists have the ability and access to technology that allows for gene editing, they are solely responsible for deciding what should be changed. From both a philosophical and an evolutionary perspective, this amount of power is dangerous. The issue of eugenics also arises within this debate. The power to alter certain genes perceived as undesirable, partnered with the goal of creating an *ideal population* takes this debate about ethics to a more dangerous level.[12] This concept of *ideal* is completely subjective and strongly fosters discrimination, which may impact specific disabled communities.[12]

Many healthcare systems worldwide operate on a basic needs system, which provides a standard level of healthcare for all members of society. The growing interest in gene editing may jeopardize equitable access. This genetic enhancement will likely be a luxury. Procedures of this nature may only be available to those who can afford it, and it is estimated that certain forms of gene therapy could cost as much as $1 Million USD.[13,14] Socio-economic class already determines who can get medical procedures in many places worldwide, and this may also apply to gene editing in the future.[13] This would further expand the opportunity gap between the rich and the poor, which is problematic.[13]

Any genomic modification has both biological ramifications and ethical considerations, and any theoretical benefits must be weighed against potential risks. Given these consequences, both for the embryo and in terms of greater societal implications, this type of genetic modification cannot be permitted.

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